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GENETICS

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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA. XII. THE AMPHIDIPLOID *RUSTICA-PANICULATA* HYBRID; ITS ORIGIN AND CYTOGENETIC BEHAVIOR[1]

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Received March 3, 1930

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INTRODUCTION

Though a great deal has been discovered as to the behavior of partially fertile interspecific hybrids, in no case has there been a complete cytogenetic analysis and explanation of the results of selfing and backcrossing such hybrids and derivatives in successive generations. And yet such an analysis is basic for in no other way can we know the stable recombination possibilities of this class of species hybrids. A really complete study of even one hybrid would not only be of great value in predicting the behavior of others, but would undoubtedly throw much light on such questions as the relationship of the species concerned, possibility of crossing over between homologous chromosomes of different species, origin of true breeding hybrid derivatives, and cause of interspecific sterility. For this purpose one must first determine (1) the meiotic behavior of the chromosomes of the hybrid; (2) the chromosome constitution of the functional male and female gametes and (3) the chromosome constitution and behavior of the F_2

[1] The author is indebted to Doctor R. E. CLAUSEN for helpful advice and interest throughout the course of this investigation.

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progeny. Obviously, in order to make much progress, the hybrid selected for study must have a rapid life cycle and ease of cytogenetic manipulation.

Accordingly, the *paniculata-rustica* hybrid has been selected for such an intensive study. It is one of many hybrids exhibiting the Drosera scheme and up to the present time is the only interspecific Nicotiana hybrid from species differing in chromosome number which although notably deficient in fertility may yet yield progeny on selfing. Although the classic example of interspecific hybridization, being among the first hybrids obtained by KOLREUTER (1759), yet relatively little progress was made in its analysis in pre-Mendelian times. As noted by FOCKE (1881) early hybridizers, especially GÄRTNER (1849), observed that in common with other hybrids its progeny exhibited reversion to the parental type and fertility. Finally EAST (1921) demonstrated that stable recombination products may be established from it. None of these studies took into account the true cytological situation.

A re-inquiry from the viewpoint of cytology was made by GOODSPEED, CLAUSEN and CHIPMAN (1926) who demonstrated that the chromosome numbers of the parents were *rustica* 24_{II} and *paniculata* 12_{II}, and that the F_1 exhibited conjugation regularly according to the Drosera scheme, $12_{II} + 12_I$. Preliminary studies of the backcross progenies were also made. More extended studies by the author (1929) revealed that the functional female gametes of the F_1 *rustica-paniculata* hybrid were in two classes, 68 percent having arisen through normal distribution of bivalents accompanied by approximately random distribution of univalents and the remaining 32 percent having approximately the somatic chromosome number of the F_1 hybrid. In the first type of distribution the average number of univalents in the viable gametes was 5.38 as compared with an expectation of 6 based on purely random distribution.

While the application of the above results to the genetic phenomena reported by EAST (1921) was not self evident, the prediction was made that the production of such a high proportion of diploid gametes must have a significant effect on the F_2 and play an important part in the production of constant *rustica* derivatives differing from the original type.

In order to complete the basic studies needed for an analysis of the subsequent generations a knowledge of the functional male gametes and viable zygotes resulting from union of such gametes with the two classes of functional female gametes above outlined is essential. The present paper reports the results of a study of (1) the progeny resulting from F_1 used as a male parent; (2) the F_2 generation; and (3) the relatively stable derivatives obtained by selfing of fertile F_2 plants.

MATERIAL AND METHODS

The variety *rustica pumila* (U. C. B. G. 169/08) was chosen to represent the species, largely because it is a small, early maturing variety more easily manipulated than larger varieties. It is our smallest variety of *rustica* and under favorable conditions may mature in 10 to 12 weeks from sowing. The strain of *paniculata* (U. C. B. G. 10/07) used is one typical of this highly stable species. Descriptions and figures of these species are contained in SETCHELL'S (1912) account of the genus. The F_1 hybrid exceeds both parents in vigor and like other *paniculata-rustica* hybrids is partially fertile. When crossed as female parent to the parental species capsules are produced containing an average of approximately 40 seeds each. One experiences much more difficulty in producing seeds by self-fertilization or by backcrossing as the male parent.

Chromosome numbers were determined by examination of aceto-carmine smears of P. M. C. Check determinations by root tip counts were made in some cases from material fixed in Navashin's modified solution and stained in iron haemotoxylin. The drawings of chromosome garnitures were made with the aid of a camera lucida. A 2 mm Leitz apochromatic oil immersion lens and ×8 aplanatic eyepieces were used in working with P. M. C., giving at table level figures magnified about 2700 diameters; with root tips, ×15 aplanatic eyepieces were used, giving figures magnified about 5100 diameters.

THE FUNCTIONAL MALE GAMETES OF F_1 *PANICULATA-RUSTICA*

In the summer of 1928, 50 flowers of a *paniculata* plant growing in the greenhouse were emasculated and pollinated with F_1 pollen. One capsule resulted containing 56 seeds, many of which were shriveled. Only two seeds germinated and the seedlings differed from each other and from *paniculata*. One died before flowering. The other was very different from *paniculata* in both flower and leaf morphology and was both self and backcross sterile. It exhibited $12_{II}+5_I$ at I-M. in the P. M. C.

The first series of backcrosses to *rustica* was made in the summer of 1928. One series of 70 pollinations resulted in 10 capsules with 29 seeds, 8 of which germinated. Six lived to flowering, at which time it was determined that two were identical with *rustica pumila*, completely fertile, and exhibited 24_{II} at I-M. The other four differed from each other and from *pumila*. Their chromosome numbers were $20_{II}+4_I$ (2), $1_{sII}+6_I$ (1) and $21_{II}+3_I$ (1).

Because of the failure to secure a really representative progeny from which to draw conclusions as to pollen transmission a final attempt was made in the summer of 1929. Two *rustica pumila* plants growing in the field were caged and great care was used in emasculation to avoid the possibility of selfing. In spite of precautions, however, a check made by leaving 20 emasculated flowers unpollinated resulted in 3 capsules, two with 3 or 4 seeds per capsule and one with a well filled capsule. Hence the control was not perfect, and there is a possibility that all capsules of the backcross which set seed may have some selfed seed. This possibility must be considered in an interpretation of backcross results. One hundred backcross pollinations, using the pollen from 5 F_1 anthers on each pistil, resulted in 49 capsules with 322 seeds, many of which were badly shrunken. Ninety-three seeds germinated and about 78 plants survived to time of flowering.

A group of 12 plants were morphologically identical with *rustica pumila*. These were examined and found to exhibit 24_{II} at 1-M. Some of these plants may actually be the result of backcrossing and carry *paniculata* chromosomes incapable of causing noticeable morphological effect when in heterozygous condition. Selfed seed was saved from some of them in order to test this last possibility. For the present, however, it is believed best to consider these plants as the result of accidental self-pollination due to faulty control.

A number of plants were severely attacked by Botrytis sp. and failed to flower sufficiently for P. M. C. studies to be made. Five of these were strikingly different from *rustica pumila* and undoubtedly would have exhibited less than 24_{II}. The results of the chromosome determinations of

TABLE 1

Chromosome numbers of the progeny resulting from rustica pumila ♀ × F_1♂.

	24_{II}	$23_{II}+1_I$	$22_{II}+2_I$	$21_{II}+3_I$	$20_{II}+4_I$	$19_{II}+5_I$	$18_{II}+6_I$
1928	2	=	=	1	2	=	1
1929	28*	3	=	1	=	3	3
Total	30	3	=	2	2	3	4

* Including one plant with $24_{II}+1_I$, which was distinct from *rustica*.

the 38 remaining plants investigated are given in table 1 under the caption 1929, together with previous determinations under the caption 1928.

At least four of the plants with 24_{II} were distinct from one another and from *rustica pumila* as shown in figure 1. The other 24_{II} plants flowered

much later than the above group of twelve plants definitely classed as *rustica pumila* but were otherwise very similar to them. They were all highly self fertile. The four distinctive 24_{II} plants and the three plants having $23_{II}+1_{I}$ at 1-M in a population of only 75 plants are very good evidence for great selectivity in favor of the 24 and 23 chromosome gametes. The distinctive plants with 24_{II} also indicate that the pollen is capable of transmitting the *paniculata* homologues as readily as the egg.

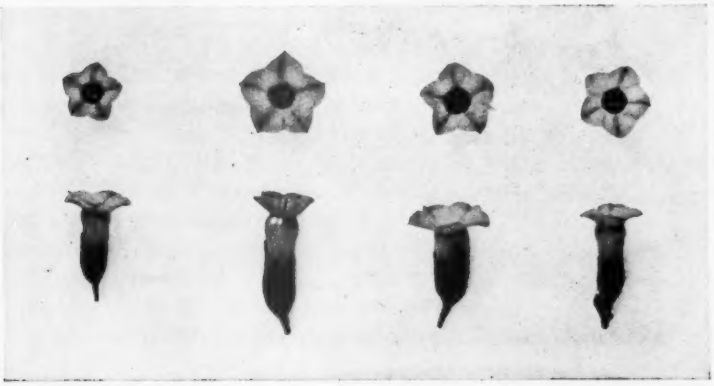

FIGURE 1.—Flowers of four 24_{II} plants of the backcross progeny of *N. rustica pumila* ♀ × F_1 *rustica-paniculata* ♂. The variation shows that F_1 pollen transmits *paniculata* homologs which express themselves when in heterozygous condition.

The other plants were highly variable and comparable to the reciprocal backcross in this respect in that no two were identical. One plant with $21_{II}+3_{I}$ and several with $18_{II}+6$ and $19_{II}+5_{I}$ were fully as sterile and probably more so than the F_1. There seemed to be no correlation between chromosome number and fertility for the plant with $21_{II}+3_{I}$ was as sterile as the one with $18_{II}+6_{I}$, and, furthermore, one plant with $19_{II}+5_{I}$ exhibited quite a high degree of fertility.

DISCUSSION

The absence of plants indicating diploid gamete formation in the above backcross progeny is very unexpected in view of the large number of them found in the progeny of the backcross, using F_1 as female, and the occurrence of a large number of amphidiploids in the F_2. However, as will be shown later, the pollen of the amphidiploid when used on *rustica* or *paniculata* fails to give viable seed, and the absence of expected class of plants

probably only indicates inability of F_1 pollen grains with somatic number of chromosomes to function on parental species rather than failure of their production. In other words, it is believed that the amphidiploids found in the F_2 are the result of union of diploid gametes, though as yet it has been impossible to demonstrate the presence of pollen grains with diploid number of chromosomes. Some evidence for this belief is found in the fact that only by hand pollination are any seeds produced by the F_1, and secondly that examination of tetrad stage in F_1 meiosis shows a few dyads. It will be of interest to determine how readily F_1 pollen functions on the amphidiploid and the type of progeny resulting.

The selectivity is also peculiar in that no plants with $12_{II}+12_I$ corresponding to those with 24_{II} were found and differs strikingly from that type of selectivity reported by THOMPSON and CAMERON (1928) in backcross of *Triticum vulgare* ♀ ×*vulgare-durum* F_1 ♂ where both the 21 and 14 chromosome grains were favored. Probably the cross of *N. paniculata* ♀ ×F_1 *rustica-paniculata* ♂ would give a progeny with most plants having approximately 12_{II}, and the peculiar results so far obtained make it necessary to make this backcross in order to properly complete the analysis.

2. THE F_2 PROGENY OF *RUSTICA-PANICULATA*

In the spring of 1928, two F_1 plants were removed to the greenhouse where only Crepis species are grown. Inasmuch as they were thus isolated from parental pollen no caging or bagging was necessary. Several hundred hand pollinations, using pollen from five anthers on one stigma, resulted in 44 capsules containing a total of 114 seeds of which 63 germinated. Several plants died in the cotyledon stage and others were severely infected with Botrytis sp. Chromosome determinations of the 37 plants which flowered revealed three classes of plants as regards chromosome number.

The first class consisting of 4 plants evidently resulted from the union of haploid gametes. Their very regular I-M behavior made determination of chromosome number very easy. They exhibited $14_{II}+10_I$ (1) and $17_{II}+7_I$ (3). They differed from each other and though generally similar to *rustica pumila* differed from it in size and shape of flowers and leaves as well as habit of growth. Though similar to the class 1 plants obtained by crossing F_1 ♀ ×*rustica* and discussed in a previous paper (LAMMERTS 1929), repeated attempts to secure seed by selfing failed and though in one plant occasional seeds were formed the capsules dropped before maturity. The plants in this class are, therefore, more sterile than the F_1 and hence are practically completely sterile.

The second class consisting of 3 plants arose from the union of diploid gametes and haploid gametes. They were in general similar to *rustica* but differed from it and among themselves in detail of flower and leaf morphology. The chromosome behavior at I-M was highly irregular as contrasted with the class 1 plants and usually the number of large chromatin elements (bivalents and trivalents) was less than 24. The irregularity in these plants was greater even than that reported for the class 2 plants, obtained in the backcross of F_1 female to *rustica*. Because of this irregularity exact chromosome determinations were not possible. Two plants had 54–55 chromosomes and the other 57–58, as determined by II-M studies. They arose then from the union of diploid gametes containing approximately 36 chromosomes with haploid gametes having 18–21 chromosomes. Inasmuch as most of the functional F_1 haploid gametes have an average of 18 chromosomes, the number of chromosomes in these class 2 plants agrees with expectation. These plants were also completely sterile and thus differed from the class 2 plants referred to above.

The third class consisting of the remaining 30 plants arose from union of diploid gametes with approximately 36 chromosomes. Inasmuch as the amphidiploid races described in detail in part 3 of this paper were derived from these plants only general statements as to their appearance and cytology need be given here. They were similar in general characters such as possession of wide, rather heart shaped, thick leaves; flowers comparable to the F_1 in tube length and to *rustica pumila* in tube width; and high degree of fertility. They were distinct from one another, however, in growth habit and detail of leaf and flower shape. The chromosome behavior at I-M was highly irregular and determination of the chromosome number of most of them was only approximate, being made from II-M. All varied around 72 chromosomes.

DISCUSSION

In a preliminary paper SINGLETON (1928) also reports the occurrence of plants having 72 chromosomes in the F_2 progeny of *N. rustica-paniculata*. The number of plants is not given but they are described as being nearly completely fertile when selfed. In a letter to the author he furthermore states that plants closely resembling *paniculata* were not recovered in his rather large F_2 population, and that the plants resulting from union of incomplete haploid gametes were very sterile. Our results are then in general quite comparable.

The absence of any plants resembling *paniculata* or of fertile *rustica* derivatives in our class 1 plants is according to expectation. In view of the

random survival of the female gametes and the large number of functional male gametes with less than 24 chromosomes the possibility of recovering 12_{II} or 24_{II} plants in the F_2 is very slight indeed. Though the data are here limited, studies of progenies resulting from selfing of a $17_{II}+7_{I}$ backcross plant show that the *rustica-paniculata* hybrid and its derivatives behave according to KIHARA'S scheme, namely, the surviving zygotes are those with a total number of bivalents and univalents equal to 24. Accordingly most zygotes arising from the union of F_1 haploid gametes do not survive. The proportion of class 2 and 3 plants should, therefore, be relatively higher than the calculated expectation based on the proportion of diploid female gametes. This is indeed the case as regards the class 3 plants, for actually about 80 percent of the F_2 are amphidiploid. Only 8 percent of the F_2 are class 2 plants, however. Most of the zygotes resulting from the union of incomplete haploid gametes and diploid gametes are therefore inviable. Though some of the F_1 sterility is due to failure of F_2 zygotes in accordance with KIHARA'S scheme, this failure obviously is not the complete explanation, inasmuch as we have already observed that the F_1 used as male parents exhibits a sterility comparable to that exhibited on selfing.

3. THE RELATIVELY STABLE DERIVATIVES OBTAINED BY SELFING FERTILE F_2 PLANTS

(*a*) *Origin and behavior of amphidiploid lines*

The class 3 plants of the F_2 progeny were rather uniformly fertile. They have been described as rather similar in general characters. Inasmuch as they exhibited much variability in chromosome number and in detail of leaf and flower morphology, it was decided to continue the study in F_3 by selecting the five F_2 plants most distinct from each other in morphology and chromosome number. A brief description of these plants and their behavior will give some idea of the variability in class 3.

Plant 28643/15 was 78 cm high; the leaves were linear and not heart shaped as the majority of F_2 class 3 plants, curled at the edges and glossy. Irregular development of leaf tissue caused areas of light and dark color. Twenty-six percent of the pollen was good as determined by aceto-carmine staining. The plant exhibited 70 chromosomes at II-M. Five capsules containing 81 seeds were sown of which 60 germinated, giving rise to F_3 line, 29001.

Plant 28643/20 was 120 cm high; the leaves were heart shaped, broader, and shorter than those of *rustica pumila*. Seventy-seven percent of the pollen was good. The plant had 72 chromosomes at II-M. One capsule

containing 160 seeds, most of which germinated, gave rise to F_3 line, 29002. This plant is perhaps typical of the F_2 class 3 plants.

Plant 28643/47 was 102 cm high; the leaves were quite long, rather narrow, and thickened due to irregular development of the leaf tissue which also gave them a mottled green color. The plant exhibited 71 chromosomes plus a fragment at II-M. One capsule containing 125 seeds, most of which germinated, gave rise to F_3 line, 29003.

Plant 28643/48 was 113 cm high and had leaves similar to those of 643/20. It had 74 chromosomes at II-M and one capsule containing 150 seeds gave rise to F_3 line, 29004.

FIGURE 2.—Comparison of (1) *N. rustica pumila*, (2) F_1 hybrid, (3) amphidiploid hybrid and (4) *N. paniculata* flowers.

Plant 28643/50 was 117 cm high. The leaves were similar in shape to those of 643/20 but more emarginate. It had 77–78 chromsomes at II-M and one capsule containing 143 seeds gave rise to F_3 line, 29005.

All these plants as well as the remaining class 3 plants were very similar in flower size and shape, though, of course, exhibiting some variability, especially in leaf morphology and chromosome number; and the selected plants were considered representative. Only one plant in the entire class, namely, 28643/15 was at all infertile. The others, though much less fertile than *rustica*, which averages about 550 seeds per capsule, were comparable with one another, averaging about 150 normal-appearing seeds per capsule. Besides these there are many shriveled seeds which fail to germinate.

The five F_3 lines resulting from these plants were comparable in every way. Furthermore, each line exhibited about the same type of variability, hardly two plants being identical. The variability was much greater than that of F_2. No constant differences were noted which could serve to distinguish one line from another. Each line had dwarf and giant types, most plants, however, being intermediate between extremes, as represented by figure 7. Comparison of the relatively constant flower type with *rustica*

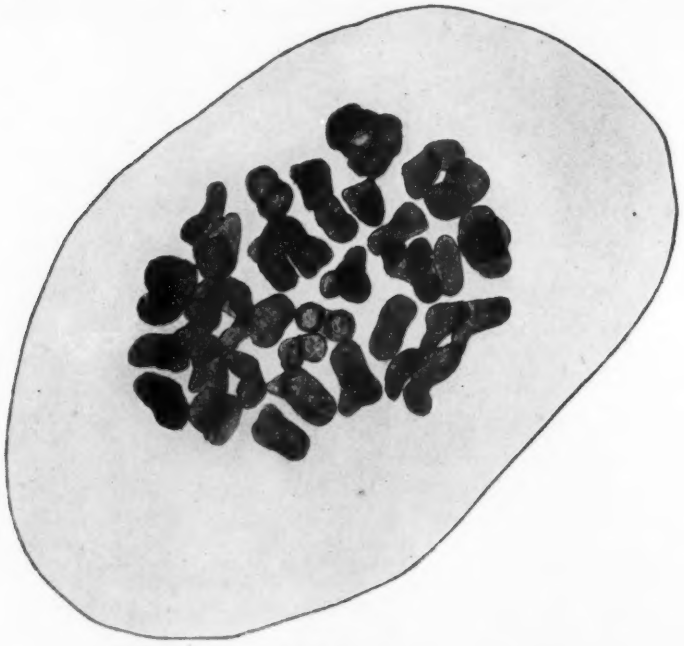

FIGURE 3.—I-M of amphidiploid plant showing $10_{IV}+16_{II}$.

pumila, the F_1, and *paniculata* is made in figure 2. It is not to be understood from this figure that the amphidiploids showed no variability in detail of flower morphology, but that such as occurred was around this type as a mode. Finally the F_3 lines resembled *paniculata* in their long flowering period, this being especially noticeable under field conditions where *paniculata*, the F_1 hybrid and the amphidiploid, remained flowering long after *rustica pumila* and its derivatives.

Cytologically the plants were very similar in their chromosome behavior. Many plants were examined and all showed very irregular behavior at I-M. Within a given aceto-carmine mount the number of chromatin elements varied from approximately 24 to 36. The cell shown in figure 3 is characteristic. It was interpreted as having $10_{IV}+16_{II}$. II-M counts checked this interpretation, inasmuch as 72 chromosomes were counted. Figure 4 shows

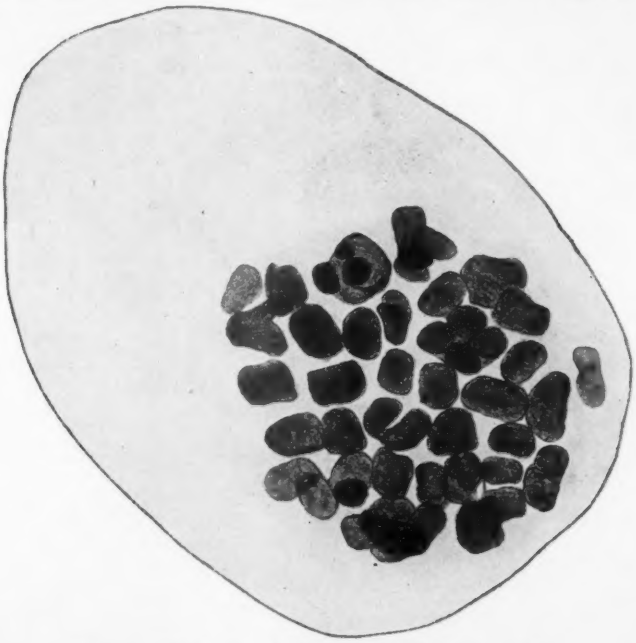

FIGURE 4.—I-M of amphidiploid plant showing $4_{IV}+30_{II}+2_{I}$.

a more unusual condition, that is, $4_{IV}+30_{II}+2_{I}$. Rarely one finds a plate showing the maximum number of quadrivalents, that is, $12_{IV}+12_{II}$. The II-M are very clear, and though it is difficult to find decisive plates, when located, they agree as to number. The determination of the five F_2 plants and F_3 plants selected for parents of the F_4 generation is usually based on counts of 5 II-M plates each. Figure 5 shows a II-M of a typical F_3 plant exhibiting 72 chromosomes.

Five F_3 plants were selected for the F_4 generation. As with the F_2 an endeavor was made to pick extreme types with the idea of establishing possible derivatives. Some idea of the appearance of these amphidiploids and

the variability exhibited by them may be obtained from reference to figures 6-10. The dwarf plant (figure 6) proved to be completely sterile. It exhibited 72_I at II-M. There were very few sterile plants and it is interesting that in spite of a numerically complete double set of chromosomes, complete sterility is possible. Three of the other plants were determined as having approximately 72 chromosomes and one (figure 10) was definitely determined as having 73 chromosomes.

The four F_4 lines arising from these plants were distinct from one another. Each line exhibited variability but not to the extent found in F_3,

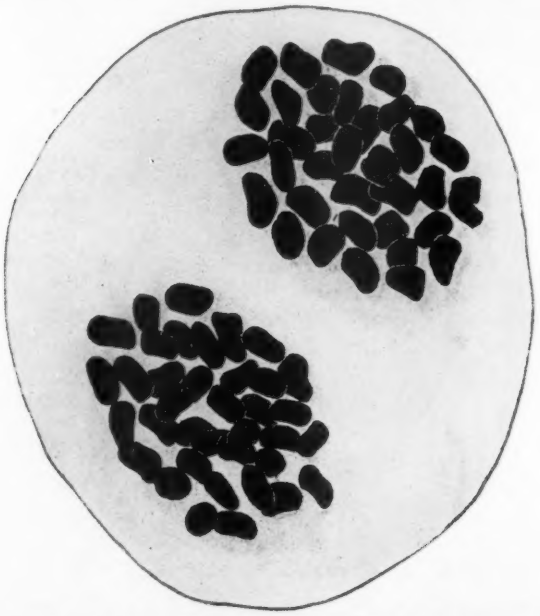

FIGURE 5.—II-M of amphidiploid plant showing 72 chromosomes.

and furthermore it was of a type distinctive for each line. In other words, four derivatives have been established from as many F_3 plants, and these show a greater degree of constancy than was found in the F_2 and F_3. They are similar in chromosome behavior to the F_2 and F_3, exhibiting a variable number of bivalents and quadrivalents. Investigations are being continued into the F_5 and further generations in order to determine subsequent behavior. The possibility of securing other more striking derivatives is also being investigated. It is planned to secure more F_3 material from F_2 seed

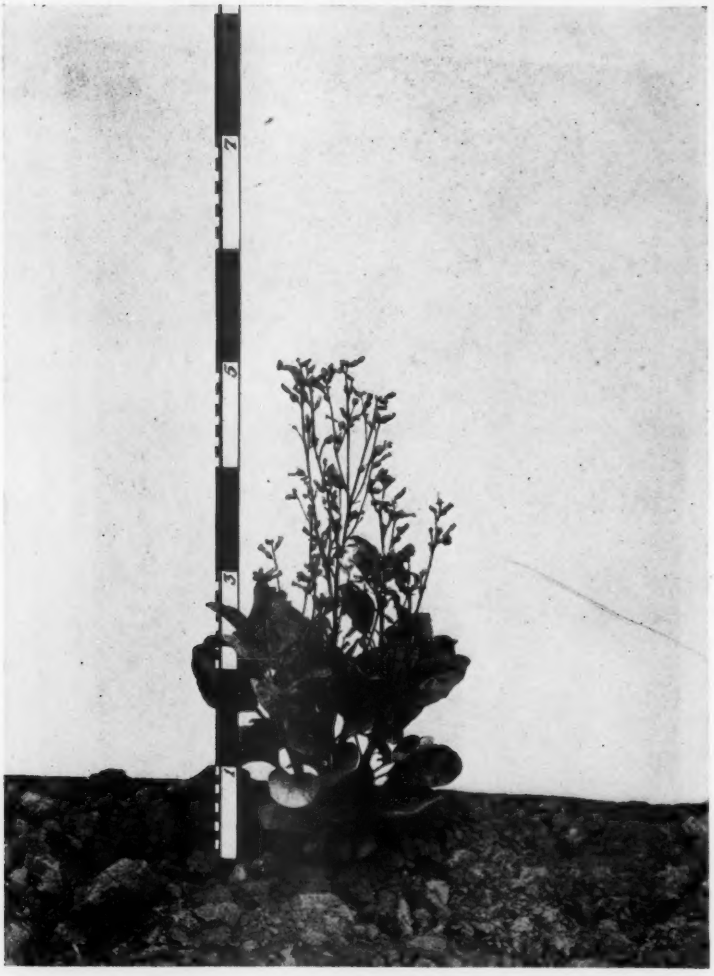

FIGURE 6.—A dwarf type plant found in an F_3 amphidiploid line. It exhibited 72 chromosomes at II-M and yet was completely sterile.

and to make selections of other plants for F_4 lines as was described above so as to get a clearer picture of the extent to which derivative lines may be built up.

(b) *Result of crossing amphidiploids with parental species*

(1) Reciprocal backcrosses to *rustica pumila*

Plant 29001 was used for making crosses with the parental species. It

FIGURE 7.—Typical F_3 amphidiploid plant. This plant exhibited 72 chromosomes at II-M.

is shown in figure 7. The chromosome number was definitely determined from a number of II-M plates to be 72.

When this plant was used as the male parent on *rustica pumila* well-filled capsules were obtained. The seed though somewhat shriveled ap-

FIGURE 8.—A striking variant found in an F_2 amphidiploid line. Note long flower tubes and narrow leaves. This plant exhibited approximately 72 chromosomes at II-M.

peared good. However, two tests resulted in only one germination from 538 seeds. The reciprocal backcross, using 29001/4 as female parent, produced well-filled capsules. From one capsule containing about 150

FIGURE 9.—A giant type F_1 amphidiploid plant exhibiting approximately 72 chromosomes at II-M.

FIGURE 10.—A giant type F_2 amphidiploid plant exhibiting 73 chromosomes at II-M.

seeds only 6 plants were obtained of which two survived to transplanting. A repeated germination gave similar results, only 8 in 168 seeds germinating.

(2) Reciprocal backcrosses to *paniculata*

When 29001/4 is used as the female parent the capsules, though at first normal in appearance, at maturity show only shriveled, chaffy seed which usually fails to germinate. In one test 8 capsules had only 4 possibly good seeds of which 2 germinated. It is as yet undetermined whether these are actually hybrid plants. Cross sections stained with haemotoxylin show that 9 days after pollination the ovules of the backcross have poorly developed endosperm and relatively smaller size than comparable ovules arising from self-pollination.

When 29001/4 is used as the male parent on *paniculata* the capsules contain only chaffy seed which failed to germinate.

DISCUSSION

The behavior of the amphidiploid on backcrossing to its parents is very unexpected in view of the known fact that zygotes arising from union of F_1 somatic gametes with parental pollen are viable and grow into the class 2 plants described in a previous paper (LAMMERTS 1929). The diploid gametes of the amphidiploid are presumably the same as those produced by the F_1, and theoretically the amphidiploid should be fertile with its parents, instead of almost completely sterile. The Raphanus-Brassica hybrid investigated by KARPECHENKO (1927) behaves similarly with its parents. This phenomenon is as yet unexplained, though several suggestions as to its cause are being investigated. From the viewpoint of phylogeny the two cases are of especial interest. If these synthetic "species" existed under natural conditions cytogeneticists would be tempted to consider the sterility they exhibit with their parents as due to chromosome incompatibility and hence infer that the species investigated were only distantly related. In other words, the behavior of these two amphidiploids with their parents shows clearly that crossability is not a satisfactory index of species relationship.

It may seem rather unexpected that the segregation of the amphidiploid is apparent for the first time in the F_3. Theoretically, however, the F_2 plants arise from somatic gametes genetically identical except in so far as non-disjunction has brought about loss or gain of chromosomes. The F_2 plants at meiosis have been shown to exhibit a variable number of quadrivalents and if these dissociated at random, gametes varying genetically would result. The F_3 plants, therefore, would vary both morphologically

and genetically and give rise on selfing to lines of distinctive variability. This has indeed actually proved to be the case and it is believed that the evidence presented proves that dissociation of the quadrivalents is not strictly preferential.

Although a number of amphidiploid hybrids have been recorded, in only a few cases have they been subjected to an extended study. The most complete studies are those of *Nicotiana digluta* (36_{II}) (CLAUSEN 1928); *Primula kewensis* (18_{II}) (NEWTON and PELLEW 1929); the Raphanus-Brassica hybrids (18_{II}) (KARPECHENKO 1927); and the Digitalis hybrids (56_{II}) (BUXTON and NEWTON 1928). These as well as the others recorded may be divided into two groups as regards origin. In one class are those which arose, not as a result of regular formation of diploid gametes by an F_1 hybrid, but rather by a chance doubling in the F_1 zygote (*Nicotiana digluta*, *Primula kewensis*) or by chance union of rarely formed diploid gametes by the F_1 as in the *Nicotiana sylvestris-Tabacum* amphidiploid (RYBIN 1928). The other class originate as a result of regular formation and functioning of diploid gametes by an F_1 hybrid and in this category may be placed the Raphanus-Brassica hybrid, and the Digitalis hybrid. Until recently it was assumed that regular formation of diploid gametes depended upon complete lack of pairing, inasmuch as cytologic studies by FEDERLEY in his Pygaera hybrids and the above hybrids were in this category (FEDERLEY 1928). Furthermore, in both the Digitalis and Brassica F_1 hybrids the only functional gametes were those having diploid or hyperdiploid chromosome numbers, while the great majority of gametes being random combinations of parental chromosomes were non-functional. In the case of the amphidiploid *rustica-paniculata* hybrid, however, the F_1 regularly exhibits the Drosera scheme, $12_{II}+12_{I}$, and many of the haploid gametes, though incomplete, are functional. Besides there are formed regularly diploid gametes which give rise to amphidiploid F_2 plants. The supposition then that somatic gamete formation is necessarily dependent upon lack of pairing can no longer be entertained.

The question of the constancy of these hybrids is of interest from the standpoint of evolutionary significance. When an amphidiploid results from doubling up in a hybrid which exhibits complete lack of pairing, there is theoretically little chance for anything but constancy and such is indeed the observed behavior in the cases so far investigated. But when an amphidiploid originates from a hybrid exhibiting pairing, quadrivalent formation with consequent segregation into numerous strains is theoretically possible. The F_1 of *P. verticillata*×*floribunda* shows 9 loosely formed pairs at I-M. In spite of this, however, *P. kewensis* usually exhibits 16 bivalents

and only one quadrivalent. As a consequence numerous derivative lines have been established, the members of which vary around a type distinct from that of another line. Apparently recombination of *floribunda* and *verticillata* chromosomes is here occurring, leading eventually to the formation of numerous derivatives, all amphidiploid as regards chromosome number.

The amphidiploid *rustica-paniculata* hybrid also has the theoretical possibility of breaking up into numerous derivatives, inasmuch as quadrivalents are formed at meiosis. So far four such lines have been obtained. Whether these will attain the constancy of the parental diploid strains or continue to vary is as yet undetermined, but the high degree of constancy in the F_4 as contrasted with the F_3 lines would lead one to expect a higher degree of constancy in successive generations.

The demonstration that in spite of F_1 pairing according to the Drosera scheme diploid gamete formation occurs regularly, leading to production of amphidiploids, is important, for it shows that interspecific hybridization as a factor in polyploidy is not limited to hybrids in which no pairing occurs.

SUMMARY

(1) Study of the backcross progeny of *N. rustica pumila* ♀ × F_1 *rustica-paniculata* ♂ shows that there is great selectivity in favor of the 24 and 23 chromosome gametes. The pollen is also able to transmit *paniculata* homologs, inasmuch as the 24_{II} plants differed from one another morphologically.

(2) The results of selfing the F_1 are in general accord with expectation based on a study of functional male and female gametes. Three classes of F_2 plants are produced: (1) sterile plants arising from union of haploid gametes and having a total of 24 bivalents and univalents; (2) sterile plants resulting from union of diploid and haploid gametes and (3) from union of diploid gametes amphidiploid plants which, because of zygotic elimination of a great proportion of the diploid zygotes, make up approximately 80 percent of the F_2 progeny.

(3) These F_2 amphidiploids as well as the F_3 show a variable number of quadrivalents, bivalents and univalents at I-M. Chromosome determinations from II-M showed that the plants used for selfing had 72 or 73 chromosomes.

(4) Four derivative lines were obtained in the F_4 which, although not constant, varied about a characteristic type. This indicates that the dissociation of the quadrivalents is not strictly preferential.

LITERATURE CITED

BUXTON, B. H., and NEWTON, W. C. F., 1928 Hybrids of *Digitalis ambigua* and *Digitalis purpurea*, their fertility and cytology. J. Genet. **19**: 269–280.

CLAUSEN, R. E., 1928 Interspecific hybridization in Nicotiana. VII. The cytology of hybrids of the synthetic species *digluta*, with its parents, *glutinosa* and *Tabacum*. Univ. California Publ. Bot. **11**: 177–211.

EAST, E. M., 1921 A study of partial sterility in certain hybrids. Genetics **6**: 311–365.

FEDERLY, H., 1928 Chromosomenverhältnisse bei Mischlingen. Proceedings of the 5th International Congress, Supplementband of the Z. indukt. Abstamm.-u. Vererblehre **1**: 195–219.

FOCKE, W., 1881 Die Pflanzenmichlinge. iv+569 pp. Berlin: Borntraeger.

GÄRTNER, C. F., 1849 Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich. xvi+790 pp. Stuttgart: Hering.

GOODSPEED, T. H., CLAUSEN, R. E., and CHIPMAN, R. H., 1926 Interspecific hybridization in Nicotiana. IV. Some cytological features of the *paniculata-rustica* hybrid and its derivatives. Univ. California Publ. Bot. **11**: 103–115.

KARPECHENKO, G. D., 1928 Polyploid hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. Z. indukt Abstamm,-u. VererbLehre. **48**: 1–85.

LAMMERTS, W., 1929 Interspecific hybridization in Nicotiana. IX. Further studies of the cytology of the backcross progenies of the *paniculata-rustica* hybrids. Genetics **14**: 286–304.

NEWTON, W. C. F., and PELLEW, C., 1929 *Primula kewenesis* and its derivatives. J. Genet. **20**: 405–467.

RYBIN, V. A., 1929 Über einen Alletetraploiden Bastard von *Nicotiana tabacum*×*sylvestris*. Ber. Deutschen Botanischen Gesellschaft **47**: 385–394.

SETCHELL, W. A., 1912 Studies in Nicotiana. I. Univ. California Publ. Bot. **5**: 1–98.

SINGLETON, W. R., 1928 A case of doubling of chromosomes in the genus Nicotiana. Anat. Rec. **41**: 93.

THOMPSON, W. P., and CAMERON, D. R., 1928 Chromosome numbers in functioning germ cells of species hybrids in wheat. Genetics **13**: 456–469.

THE GROWTH OF THE GONADS IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

As shown by DOBZHANSKY and BRIDGES (1928), triploid intersexes in Drosophila may be considered as individuals developing up to a certain moment as males and thereafter as females. The earlier or later occurrence of the turning point in the development of an intersex determines the production of an individual manifesting relatively more male or relatively more female characteristics. If the turning point occurs early in the development, intersexes are produced having more female and less male characteristics. Conversely, if the turning point occurs late, the resulting intersexes become more male-like. In the extreme cases, that is, in cases when the turning point lies very early or very late in the development, intersexes are produced which are very similar to normal females or to normal males in appearance.

On the basis of these data BONNIER (1929) suggested the possibility of finding in the development of the normal females and males a phenomenon corresponding to the turning point of intersexes. The normal sexes in Drosophila differ from each other and from the intersexes by the altered balance of the female-determining and the male-determining factors. According to BRIDGES (1925), the female-determining factors are localized predominantly in the X-chromosomes, while the male-determining factors are localized mainly in the autosomes of Drosophila. Since both the X-chromosomes and the autosomes are present in the nuclei of normal females and males, both female-determining and male-determining factors are present in the germ-plasm of the normal sexual forms. Normal males may be, therefore, considered as individuals in which the turning point in

the development does not occur until the imaginal stage is reached, or until the end of their life. On the other hand, a turning point might be present in the earliest development of the normal females.

In order to secure data which might have some bearing on the problem raised by BONNIER, a study of the development and of the rate of growth of the gonads in females and males of Drosophila was undertaken. The size of the gonads was measured in larvae of different ages, beginning with those just hatched from the eggs, then through stages fully mature, and in young pupae. In older pupae the rapid differentiation of the tissues composing the gonads, and the alteration of the form of these organs make measurements difficult for the accurate description of the processes of growth taking place at that time.

MATERIAL AND METHODS

The growth of the gonads has been studied in wild-type flies belonging to the "Florida" stock of *Drosophila melanogaster* which was brought to Leningrad by Professor H. J. MULLER in 1922. Since the studies of growth require a thorough control of the age of the material under investigation, pains were taken to secure larvae and pupae of exactly known age that had developed under the same environmental conditions.

Fertilized females, from 4 to 8 days old, were placed in glass tubes containing a slide covered with a thin film of culture medium (raisin-agar sprinkled with a solution of commercial yeast). After 2 to 3 hours the slides with eggs deposited on the surface of the medium were removed from the tubes, and the eggs were carefully transferred one by one to the surface of a new slide covered by a still thinner film of raisin-agar, which was sufficiently homogenous and transparent to make the eggs easily noticeable. These slides were kept in a moist chamber in an incubator at 27.0 ± 0.3°C.

In order to obtain larvae of the same age, the slides were examined after 18 to 20 hours, and the larvae hatched from the eggs in the course of this time were discarded, since freshly laid eggs sometimes contain embryos in very different stages of development. Even fully formed larvae may be present in the freshly laid eggs (HUETTNER 1923). Thereafter the slides were examined regularly at one-hour intervals, and the freshly hatched larvae transferred to small crystallizing dishes provided with a standard amount of food (25cc of raisin-agar and 3 drops of 20 percent solution of yeast). Not less than 40 and not more than 50 larvae were placed in one crystallizing dish.

The further development of the larvae took place in the same incubator at 27°. Every 12 hours some of the larvae were extracted from the food, dissected, and the size of the gonads was measured. Their age was, therefore, known with an error not exceeding $\pm\frac{1}{2}$ hour.

The age of the pupae was controlled by a similar method. Cultures containing fully grown larvae were inspected at one-hour intervals, and freshly formed pupae were transferred individually to the surface of moist filter-paper. The development of pupae took place also at 27°C. Every 6 hours some of the pupae were dissected and the size of their gonads measured.

The dissection of the larvae and pupae was performed under a binocular microscope, in a drop of physiological salt solution, by means of sharp preparation-needles. The gonads sufficiently isolated from the fat-body were measured in units of an eyepiece-micrometer. Only one gonad was measured in each individual. Since the gonads of larvae and young pupae have the form of a nearly ideal ellipsoid, two measurements are sufficient for the characteristic of the size of a gonad. These measurements are the longer and the shorter diameters of the ellipsoid, and were made on each of the gonads studied. In the following tables the dimensions of the gonads are represented in terms of the units of the eyepiece-micrometer, one unit being equal to 1.12μ.

GENERAL CHARACTERISTICS OF THE LARVAL AND PUPAL GONADS

In the freshly hatched larvae of both sexes the gonads already represent well formed separate bodies which may be isolated from the surrounding tissues. The larval gonads lie in the body cavity surrounded by the fat-body, with which they are connected by very thin tracheal branches. The gonads are easily noticeable among the masses of fat-body because the tissue of the gonads is transparent and has a high refractive index, while the fat-body is not so transparent and is gray in color.

The sex of the gonads can be determined in freshly hatched larvae. The female gonads are considerably smaller in size than the male gonads, and are also different in their relation to the fat-body. The female gonad is covered by the fat-body tissue, while the male gonad lies more free, being only bordered by the fat-body. The indifferent stage of the development of the gonads can not, therefore, be found in larvae. It remains to be determined whether there is a still earlier stage at which the sex of a gonad can not be distinguished either by an anatomical or by a histological investigation.

The above description of the gonads of the freshly hatched larvae fits almost equally well for the gonads of the larvae of all the older stages. The

form of the gonads becomes more oblong-ellipsoidal with age. This is especially true in respect to the male gonad. Besides this and the rapid increase in size the gonads undergo little or no change during the whole larval stage. In the 6-hour larvae the male gonads are already so large that they begin to be seen through the body-wall of the living larva. This fact gives the possibility to determine the sex of the larvae without dissection. The accuracy of such determination has been checked by classifying the sex of 6 to 10 hour larvae and raising the females and males in separate bottles. The sex of the mature flies which appeared in these bottles was in accord with the expectation.

The genital ducts (oviducts, *vasa deferentia*) are represented in larvae by an imaginal disc lying ventral to the end of the intestine. No connection of any kind between the imaginal discs and the gonads is observable in larval stages. After the pupation the imaginal discs begin to grow very rapidly, being gradually transformed into the genital ducts. The structure of the gonads also undergoes profound changes in the pupal stage. The rudiments of the egg-strings are noticeable in the female gonad of very young pupae. In 6 to 12 hour pupae these rudiments are plainly visible. True egg-strings are, however, formed much later, in the second half of the pupal period. At 36 to 42 hours after pupation the female gonads become attached to the oviducts. In the male gonad there is observed a rapid differentiation of the tissue composing the gonad. In the 24-hour pupae bundles of developing spermatozoa may sometimes be observed in the male gonads. In the 30-hours old pupae the male gonads begin to lose their regular ellipsoidal shape; their longer diameter begins to grow much faster than the shorter diameter, the gonads become oblong, and gradually assume the spiral form characteristic of the adult testis. At the same age the male gonads become attached to the *vasa efferentia*.

Since after pupation the histological differentiation of the tissues composing the gonads becomes very rapid, the rate of growth of the female and male gonads is strictly comparable only in the larvae and very young pupae. I studied the dimensions of the gonads up to the 30-hour pupae. In still older stages the measurements of the two diameters of the gonads become insufficient for even an approximate description of the process of growth taking place at that time.

THE GROWTH OF THE LINEAR DIMENSIONS OF THE GONADS

As mentioned above, the size of the longer and the shorter diameters of the female and the male gonads was measured at 12-hour intervals in the larval stage and at 6-hour intervals in the pupal stage (pupation occurs at

TABLE 1

Growth of the linear dimensions of the female gonads.

	AGE IN HOURS	LONGER DIAMETER				SHORTER DIAMETER				n
		$M \pm m$	σ	Lim	Diff.	$M \pm m$	σ	Lim	Diff	
Larvae	0	2.88±0.10	0.51	2 –4		2.52±0.08	0.39	1½–3		25
					0.16±0.14				0.16±0.10	
Larvae	12	3.04±0.09	0.44	2 –4		2.68±0.09	0.46	2 –3		25
					0.96±0.17				0.72±0.14	
Larvae	24	4.00±0.14	0.69	3 –5		3.40±0.13	0.63	2 –5		25
					0.20±0.20				0.40±0.17	
Larvae	36	4.20±0.13	0.57	3 –5		3.80±0.10	0.49	3 –5		25
					0.44±0.17				0.56±0.14	
Larvae	48	4.64±0.12	0.62	4 –6		4.36±0.10	0.48	4 –5		25
					0.96±0.17				0.72±0.14	
Larvae	60	5.60±0.14	0.69	5 –7		5.08±0.08	0.39	4 –6		25
					1.92±0.20				1.24±0.14	
Larvae	72	7.52±0.14	0.70	6 –9		6.32±0.12	0.61	5 –8		25
					1.92±0.24				1.60±0.22	
Larvae	84	9.44±0.20	0.98	8–12		7.92±0.19	0.97	6–10		25
Pupae	0	13.12±0.14	0.71	12–14		9.88±0.14	0.71	9–12		25
					3.84±0.24				1.84±0.22	
Pupae	12	16.96±0.21	1.04	15–19		11.72±0.17	0.87	10–14		25
					0.02±0.28				1.08±0.24	
Pupae	18	16.98±0.19	1.23	15–19		12.80±0.18	1.18	11–15		44
					0.18±0.32				1.88±0.28	
Pupae	24	17.16±0.25	1.28	15–20		14.68±0.23	1.16	12–18		25
					0.58±0.35				1.26±0.32	
Pupae	30	17.74±0.25	1.46	15–21		15.94±0.22	1.30	14–20		34

TABLE 2

Growth of the linear dimensions of the male gonads.

	AGE IN HOURS	LONGER DIAMETER				SHORTER DIAMETER				n
		$M \pm m$	σ	Lim	Diff	$M \pm m$	σ	Lim	Diff	
Larvae	0	4.30±0.06	0.35	4 –5		3.89±0.05	0.27	3 –4		30
					1.58±0.14				1.27±0.10	
Larvae	12	5.88±0.13	0.65	5 –7		5.16±0.11	0.54	4 –6		25
					2.00±0.20				1.00±0.17	
Larvae	24	7.88±0.17	0.86	6–10		6.16±0.12	0.61	5 –7		25
					3.16±0.22				2.24±0.17	
Larvae	36	11.04±0.16	0.82	10–13		8.40±0.14	0.69	7–10		25
					2.92±0.24				2.84±0.20	
Larvae	48	13.96±0.19	0.94	12–16		11.24±0.13	0.65	10–12		25
					3.48±0.32				2.56±0.28	
Larvae	60	17.44±0.26	1.30	15–19		13.80±0.25	1.23	11–16		25
					3.84±0.39				2.52±0.41	
Larvae	72	21.28±0.29	1.46	19–25		16.32±0.33	1.64	13–20		25
					4.40±0.23				4.16±0.45	
Larvae	84	25.68±0.38	1.89	22–29		20.48±0.30	1.50	18–23		25
Pupae	0	27.80±0.34	1.72	25–30		21.24±0.31	1.53	18–23		25
					1.83±0.43				2.68±0.42	
Pupae	12	29.63±0.28	1.64	27–33		23.92±0.29	1.71	20–27		35
					2.13±0.47				1.81±0.41	
Pupae	18	31.76±0.38	2.04	28–37		22.11±0.29	1.56	20–26		29
					1.76±0.54				1.17±0.46	
Pupae	24	33.52±0.38	1.92	30–37		23.28±0.36	1.81	20–28		25
					4.28±0.58				0.48±0.56	
Pupae	30	37.80±0.44	2.22	35–43		23.76±0.42	2.12	20–30		25

the temperature used at $88.6 \pm .07$ hours after hatching from the eggs, the extrusion of the anterior spiracles being taken as the moment of pupation).

The results of the measurements are presented in tables 1 and 2. Besides the mean values and their mean errors ($M \pm m$) there are included in these tables also the observed limits of variation (Lim), the standard deviations (σ) and the number of individuals studied (n). The columns marked "Diff" present the differences observed between the size of the longer (or the shorter) diameter of the gonads observed in successive ages, that is, the amount of increase of the size of the respective diameter during the given interval of time. As is shown by the figures included in tables 1 and 2, these differences are in most cases larger than their trebled errors, that is, they are statistically significant. This rule, however, does not hold for

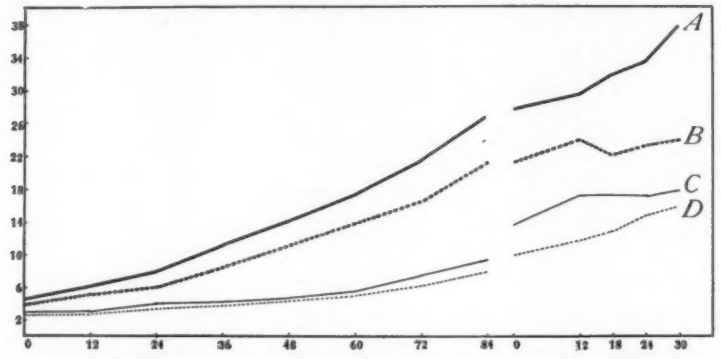

FIGURE 1.—Growth of the two diameters of the gonads. A, longer diameter of the male gonad; B, shorter diameter of the male gonad; C, longer diameter of the female gonad; D, shorter diameter of the female gonad. Horizontal axis—age in hours; vertical axis—size in units of the micrometer.

the younger ages of the female larvae, where the 12-hour intervals give in most cases statistically uncertain increase of the size of the gonads. This fact may be due either to the slow rate of growth of the female gonads in the beginning of the larval stage, or to the inaccurate determination of the size of the gonads. Indeed, the young female gonads are so small that it is difficult to measure them as accurately as the male gonads or the older female gonads.

Data on the growth of the female and male gonads may be represented also in the form of the curves shown in figure 1. The shape of these curves suggests that the rate of growth of the female gonads is somewhat different from the rate of growth of the male gonads. The male gonads of the freshly

hatched larvae are markedly larger than the female gonads of the same age. Furthermore, the size of the male gonads increases more rapidly than that of the female gonads. The curves of growth of the female gonads run nearly parallel to the horizontal axis of the diagram for the first half of the duration of the larval stage. In older larvae the increase of the size of the female gonads proceeds more rapidly. After pupation the shorter diameter of the male gonads grows less rapidly than the longer diameter. This fact is demonstrated by the rapid divergence of the curves representing the growth of the two diameters of the male gonads after pupation. The begin-

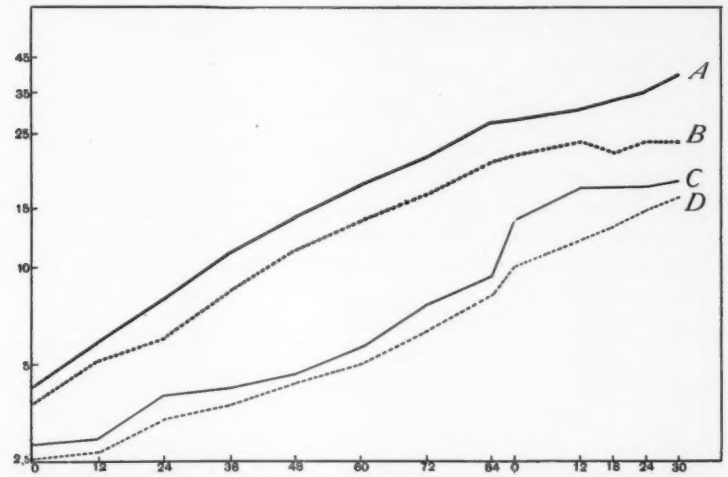

FIGURE 2.—Logarithmic curves representing the growth of the two diameters of the gonads. A, longer diameter of the male gonad; B, shorter diameter of the male gonad; C, longer diameter of the female gonad; D, shorter diameter of the female gonad. Horizontal axis, age in hours.

ning of this divergence corresponds apparently with the beginning of the rapid differentiation of the tissues composing the male gonads (see above). No such sharp divergence is observed for the rate of growth of the longer and the shorter diameters of the female gonads.

The differences in the rate of growth of the female and the male gonads become more evident if the growth is represented by logarithmic curves (figure 2); in this figure the vertical axis represents the logarithms of the diameters of the gonads. The curves of growth of the longer and the shorter diameters of the male gonads are similar in shape. The angle formed by these curves with the horizontal axis of the diagram progressively diminishes with age and finally tends to approach zero (at least for the

shorter diameter). This fact shows the progressive decrease of the rate of growth of male gonads with age.

It is well known that the rate of growth of a whole organism or of a separate organ tends to decrease with age. The curves of growth of the female gonads are different. The angle formed by these curves and the horizontal axis of the diagram increases with age at least during the whole larval stage. That is to say, the rate of growth of the female gonads shows a progressive increase with age instead of a decrease. The increase of the rate of growth with age is observed in general less frequently than the opposite condition. This fact suggests that the growth of the female gonads takes place under some influences which distort the usual shape of the growth curve.

THE GROWTH OF THE VOLUME OF THE GONADS

The male as well as the female gonads have a regular ellipsoid shape during the whole larval and the beginning of the pupal stage. The volume of an ellipsoid can be easily calculated according to the formula $V = 4/3\pi a\, b^2$, where a is the longer and b is the shorter half-axis of the ellipsoid. The volumes of the female and the male gonads calculated according to this

TABLE 3
Growth of the volume of the gonads.

AGE IN HOURS	LARVAE							
	0	12	24	36	48	60	72	84
Females	9.57	11.43	24.20	31.74	46.16	75.6	157.2	309.9
Males	33.88	78.79	156.49	407.67	923.10	1738.1	2966.1	5636.9
	PUPAE							
	0	12	18	24	30			
Females	670.2	1219.2	1455.9	1935.3	2358.9			
Males	6563.3	8869.4	8792.6	9507.0	11117.0			

formula are presented in table 3. Figure 3 shows the logarithmic curves representing the growth of the volume of the gonads.

The consideration of the curves shown in figure 3 leads to the same conclusions as the consideration of those shown in figure 2. The angle formed by the curve of growth of the male gonads with the horizontal axis of the diagram decreases rather regularly with age. The angle between the curve of growth of the female gonads and the horizontal axis of the diagram does not show the tendency to decrease with age, but increases pro-

gressively, at least until the pupal stage is reached. Taken as a whole, the curve of growth of the male gonads is convex toward the upper side of the diagram, while the curve of growth of the female gonads is somewhat convex toward the lower side of the diagram. In other words, the rate of growth of the male gonads decreases with age, while the rate of growth of the female gonads increases with age, at least until the pupal stage is reached. Only after pupation does the rate of growth of the female gonads cease to increase, and subsequently it begins even to decrease with age.

It may be suggested that the difference in the shape of the growth curves observed between the female and the male gonads is due to the fact that

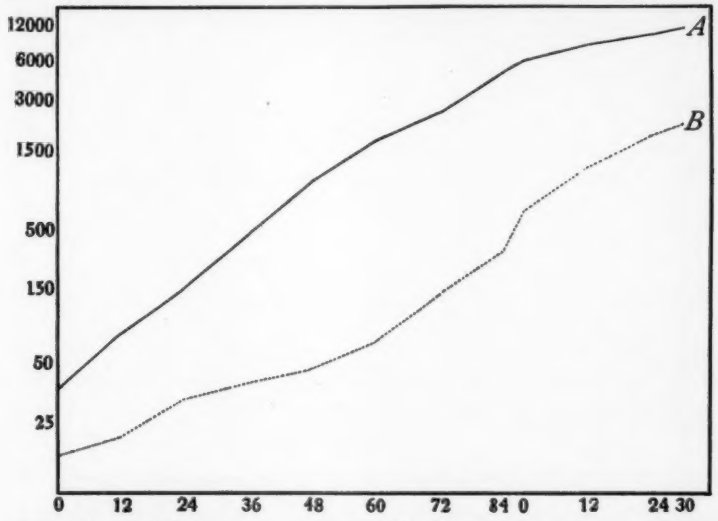

FIGURE 3.—Logarithmic curves representing the growth of the volumes of the gonads. A, male gonad; B, female gonad. Horizontal axis—age in hours.

the absolute size of the male gonads is larger than that of the female gonads. Such an explanation is, however, unsatisfactory. If the volume of the female and male gonads observed in 30-hour old pupae is taken as 100, and the volumes observed in other stages are expressed in percentages of this value, the curves of growth assume the shape shown in figure 4. These curves show clearly that the rate of growth of the female gonads is suppressed in the beginning of the larval period and becomes more intensive toward its end.

In order to ascertain once more the existence of the inferred difference between the rate of growth of the female and the male gonads the formula

of the rate of growth proposed by SCHMALHAUSEN (1928) may be used. According to this formula the rate of growth (C_v) of the volume of an organ during the time interval $t_1 - t$ is equal to:

$$C_v = \frac{\lg v_1 - \lg v}{0.4343\ (t_1 - t)},$$

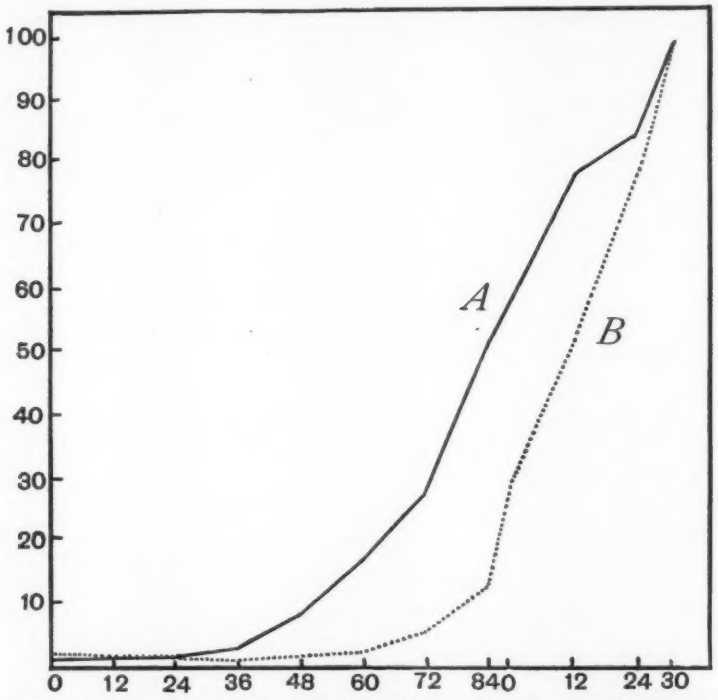

FIGURE 4.—Volume of the gonads in different ages (horizontal axis), represented in percentages of the volume attained at 30 hours of the pupal life (vertical axis). A, male gonad; B, female gonad.

TABLE 4

Coefficients (C_v) of the rate of growth of the volumes of the gonads.

AGE IN HOURS	LARVAE							PUPAE	
	0-12	12-24	24-36	36-48	48-60	60-72	72-84	0-12	12-24
Females	22.9	40.6	25.2	27.9	31.4	40.0	37.8	34.9	30.4
Males	44.6	38.1	50.0	43.4	36.1	32.7	36.5	25.9	20.6

where v and v_1 represent the volumes of the organ at the moments t and t_1 respectively. The values of C_v for the growth of the female and male gonads are shown in table 4 and figure 5. During the first 24 hours of the larval life the value of C_v fluctuates irregularly. The significance of these fluctuations is, however, doubtful, since the accuracy of determination of the size of the gonads is at its lowest just in these early stages of larval development. But beginning with the 24-hour old larvae the rate of growth of the male gonads steadily decreases, while the rate of growth of the female gonads increases with age. At the beginning of the development the rate of growth of the male gonads is higher than that of the female gonads; at the end of the larval period and in the pupal period the rate of growth

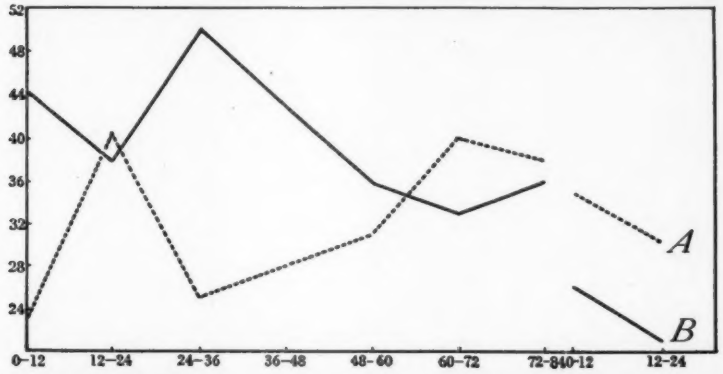

FIGURE 5.—Coefficients of the rate of growth (C_v). A, male gonad; B, female gonad. Horizontal axis—time intervals. Vertical axis—coefficients C_v.

of the female gonads exceeds that of the male gonads. After pupation the rate of growth of the female gonads begins to decrease with age, being, however, higher than the rate of growth of the male gonads.

SUMMARY

1. The sex of the gonads of freshly hatched larvae can be distinguished with complete certainty. The male gonads are larger in size than the female gonads, and show a different relation to the surrounding fat-body tissues.

2. The rate of growth of the diameters and of the volumes of the gonads has been studied. In males the rate of growth gradually decreases with age. In females the rate of growth of the gonads increases with age during the larval life. After the pupation there is observed a decrease of the rate of growth also in females.

3. During the first half of the larval stage the rate of growth of the female gonads is lower than the rate of growth of the male gonads. In the second half of the duration of the larval stage and in pupae the conditions are reversed.

LITERATURE CITED

BONNIER, G., 1929 The quantitative theory of sex. Amer. Nat. **63**: 189–190.

BRIDGES, C. B., 1925 Sex in relation to chromosomes and genes. Amer. Nat. **59**: 127–137.

DOBZHANSKY, Th., and BRIDGES, C. B., 1928 The reproductive system of triploid intersexes in *Drosophila melanogaster*. Amer. Nat. **62**: 425–434.

HUETTNER, A. F., 1923 The origin of the germ cells in *Drosophila melanogaster*. J. Morph. **37**: 385–428.

SCHMALHAUSEN, I., 1928 Das Wachstumsgesetz und die Methode der Bestimmung der Wachstumskonstante. Arch. EntwMech. Org. **113**: 462–519.

STUDIES ON SEX DETERMINATION AND THE SEX CHROMOSOME MECHANISM IN SCIARA[1]*

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INTRODUCTION

Sciara coprophila Lint., like most species of this genus thus far studied (METZ 1928), characteristically gives "unisexual" progenies. That is, the offspring from any pair mating are all or practically all of one sex, either males or females. Earlier papers have shown that the sex of the progeny as a whole is determined by the female (MOSES and METZ 1928) and that the genetic basis responsible for "sex of progeny" is inherited in a simple Mendelian fashion (METZ and MOSES 1928). It was shown also in the latter paper that the female-producing female breeds as if heterozygous, and the male-producing female as if homozygous recessive, for the gene or gene complex responsible for "sex of progeny."

Subsequently it was shown that the sex of the individual fly, as distinguished from that of the progeny as a whole, appears to be dependent on an ordinary XX-XY sex chromosome mechanism, the male being XY and the sperms, therefore, being "sex determining" (METZ and ULLIAN 1929). This conclusion is based on genetic evidence, no cytological distinction between X and Y having been detected thus far. Since a male may give both sons and daughters in large numbers (when mated to two or more

[1] This investigation has been aided by a grant from the NATIONAL RESEARCH COUNCIL, Committee for Research in Problems of Sex.

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females), it is assumed that he produces X-bearing and Y-bearing sperms in equal numbers.

The latter condition is harmonized with the production of unisexual progenies by experiments which indicate that a selective elimination or inactivation of sperms takes place (METZ 1929). These experiments lead to the conclusion that in the female-producing female the selective action is such that the eggs are fertilized only by X-bearing sperms, while in the male-producing female only the Y-bearing sperms function. Thus the agent responsible for "sex of progeny" apparently finds expression in selective fertilization (see Discussion). Speaking broadly, therefore, we may say that sex is "determined" here by two factors or complexes, the one acting

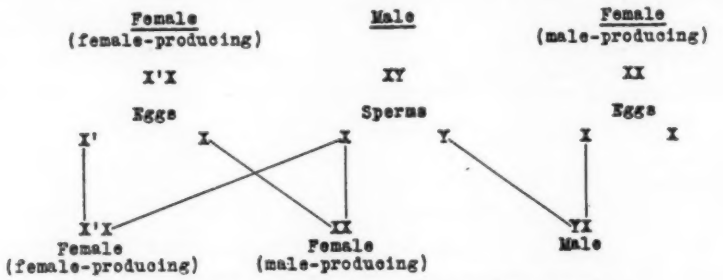


FIGURE 1.—Diagram illustrating mode of sex determination in Sciara.

directly and the other indirectly. The sex of the individual fly depends directly on the type of sperm fertilizing the egg; but the type which will so function depends in turn on the zygotic constitution of the female producing the egg.

It is the purpose of the present paper to consider evidence which shows that both of these factors or complexes are transmitted by the sex chromosome pair and that, in this respect at least, we are dealing here with three different kinds of sex chromosomes. The accompanying diagram (figure 1) will serve to illustrate this feature and to summarize the interpretation of sex determination developed below. A brief consideration of this interpretation will facilitate the presentation of data.

The evidence indicates that the male is XY, that the male-producing female is homozygous for the X found in the male, thus being XX, and that the female-producing female has one X of this kind, together with one which differs at least in respect to the agent responsible for sex of progeny. The latter is designated X′. The X′ chromosome, or the portion of it considered here, ordinarily descends directly from mother to daughter,

thus being sex-limited in its transmission somewhat like the Y-chromosome, except that it goes to only half the daughters. The X-chromosome, on the other hand, passes back and forth between the males and both kinds of females.

Interest attaches especially to the constitution of the X′ chromosome as compared with that of X and Y, and especial efforts are being made to secure a genetic analysis which will make such a comparison possible. At present the evidence is limited to the following points: (1) X′ and X have similar effects on the morphological characteristics of the wild-type fly, as indicated by the fact that the two types of females look alike, and that when X′ is substituted for X in an "exceptional" male no striking difference results. (2) X′ carries the normal allelomorphs of the two known sex linked genes. (3) No crossing over has been detected thus far between X′ and X, whereas crossovers between X and X have been obtained. These features will receive further consideration below.

In this connection it should be noted that the sex chromosomes considered here are not to be confused with the large androsomes or "male-limited" chromosomes of Sciara, found only in males (METZ, MOSES and HOPPE 1926). The latter chromosomes apparently have no sex-determining function.

For aid in connection with the present work the writers are indebted to Mrs. SILKA S. ULLIAN, who found the mutant character "swollen" and carried out the first experiments with it.

METHODS

The unusual mode of reproduction exhibited by Sciara imposes certain limitations, as well as offering certain advantages, as regards breeding technique.

For instance, in the present species (and many others of the genus) stocks or strains cannot ordinarily be secured from single wild females; each must come from at least two—a female-producer and a male-producer. Likewise, propagation of the stocks cannot be carried on by means of brother-sister matings, nor can such matings be used regularly in the experiments following crosses. F_2 progenies, therefore, must ordinarily be secured by mating sisters from one F_1 progeny to brothers from another such progeny.

It has been found in practice that even with these limitations on close inbreeding, fertility is rapidly reduced in inbred stocks, with the result that frequent out-crossing is required. Present indications are that fertility is restored by crossing with laboratory stocks as well as with wild ones,

which suggests that loss of fertility has a genetic basis. If so, this in turn suggests a possible advantage in the type of reproduction found here, which clearly restricts inbreeding. These features, however, have not been tested critically enough, as yet, to rule out environment as the causal agent. In any case the practical result is an inability to keep any stock very long without out-crossing. The "Eastern" stock referred to below, for example, is not from one source, but is from Eastern United States sources as distinguished from the "California" stocks mentioned.

It is doubtful whether geographic sources have much significance in this species, however, except within broad limits, for the flies are common in greenhouses and the larvae are readily transported long distances in the soil or decaying matter in shipments of plants. Thus far we have detected no genetic differences between flies of this species from Massachusetts, New York, Michigan and California.

Owing to the fact that the flies in any progeny mature almost simultaneously, and that the adults are very short lived (compare METZ 1929) it often happens that the particular type of mating desired cannot be made because the necessary males and females are not both available at the same time. The effect of this will be noticed in some of the experiments recorded below, particularly where wild-type males were used when narrow or swollen would have been preferable.

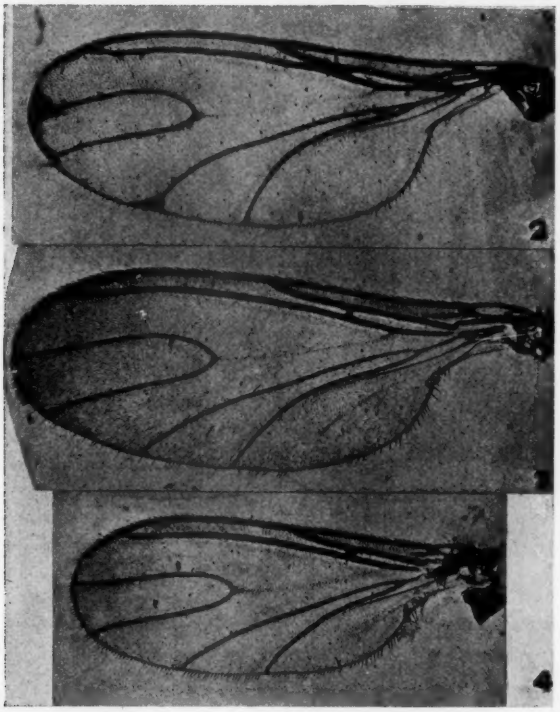
The general technique of handling the flies and the culture methods used here are not essentially different from the well known procedures used with Drosophila. The food, however, is very different. That being used at present consists of agar and leaf mold, together with commercial yeast.

TERMINOLOGY

In addition to the sex chromosome symbols mentioned above (X, X′ and Y) the following symbols are used in this paper: X*s* and X*n* indicate ordinary X-chromosomes (as distinguished from X′) carrying the genes for swollen and narrow, respectively. Thus X′X*s* represents the constitution of a female-producing female in which the X carries swollen. The symbol + is used to represent the wild-type flies as distinguished from those exhibiting mutant characters.

Since the sex of the progeny as a whole is inherited in a definite fashion it is treated as a distinct character, referred to as "sex of progeny." Females giving male progenies are designated *male-producing* or *androgenic*, and those giving female progenies as *female-producing* or *gynogenic*. As noted in earlier papers, the usual type of reproduction in Sciara, in which "unisexual" progenies are produced, is replaced in certain species and races

by a type characterized by bisexual progenies (see below, p. 238). These types of reproduction are designated *monogenic* and *digenic*, respectively.[2] The same terms may also be applied to the two corresponding types of females (that is, those giving "unisexual" progenies and those giving bisexual progenies).



FIGURES 2–4.—Photographs of wings of *Sciara coprophila*. Figure 2, swollen. Figure 3, wild-type. Figure 4, narrow. Figures 3 and 4 are from brothers, showing the usual difference in size between the wild-type and narrow wings.

Another term which may be suggested at this time is the term *androsome*, applied to the large chromosomes found only in males.[3] These chromosomes were previously referred to as "sex-limited" or "male-limited."

[2] We are indebted to Professor E. B. WILSON for these terms.

[3] See Addendum.

Technically the ordinary Y chromosome should also be included under this head, although it is not in the present paper.

As previously noted, the "male progenies" in these flies often contain a few "exceptional" females, and the "female progenies" a few "exceptional" males. In the present paper the term "exceptional" is used in this sense; it refers to the sex of the fly and not to any exceptional genetic characteristics.

THE SEX LINKED CHARACTERS SWOLLEN AND NARROW

The two mutant characters used in this study are "swollen" and "narrow." A brief description of these, together with an account of their origin, is given below.

Swollen

In swollen flies the wing veins are swollen or thickened in various ways (figure 2). The tips of the M and Ca veins are regularly thickened, delta like, and often the R and R*s* veins are irregularly swollen, lumpy or more or less fused together throughout their entire length.[4] Sometimes the whole wing is distorted by the swellings or by vesicles. Apparently the character is readily influenced by modifiers, since it is much more extreme in some lines than in others. Swollen has no noticeable effect on the viability of males, but it apparently reduces that of females materially and also prolongs their period of development so that they emerge later than their wild-type sisters.

Swollen arose in a laboratory culture of an "eastern" line (compare METZ and ULLIAN 1929).

Narrow

The primary characteristic used in the classification of narrow is the small size of the wings and their somewhat narrow shape (figure 4). The entire fly appears to be affected to some extent, however, as indicated by small body size, shorter antennae, shorter legs and poor viability. Both sexes hatch late, appear in relatively small numbers, are short lived and are much less fertile than their wild-type sibs.

Narrow arose in laboratory stock. It was first observed, by METZ, in two sister cultures in the F_2 from a cross between swollen and a bisexual ("digenic") strain (considered elsewhere) derived from Riverside, California stock (2860). The results indicated that the mutation occurred in the X chromosome from the California strain.

[4] The nomenclature of venation is that used by JOHANNSEN (1912).

INHERITANCE OF SWOLLEN

The inheritance of swollen has been considered briefly in an earlier paper (METZ and ULLIAN 1929) in which it was shown that the character behaves like a typical sex linked recessive. Narrow has not been discussed previously.

Since the inheritance of these two characters forms the basis upon which the above mentioned interpretation of sex determination rests, it will be considered here in some detail. The account of the early experiments with swollen will be found to differ in certain respects from that given in the paper just mentioned. This is due to several facts, discussed below. It need only be noted here that the difficulties from this source served for a long time to conceal the true association between "sex of progeny" and the other sex linked characters.

Swollen is inherited according to the scheme shown in figure 5. When swollen males are out-crossed to unrelated wild-type females the offspring are all wild-type. When the F_1 females are backcrossed to swollen males

1. *Wild-type female* × *swollen male.*

F_1. All wild-type.

2. *Backcross, F_1 female* × *swollen male.*

Male progenies	*Female progenies*
Wild-type and swollen	Wild-type and swollen
1:1 ratio	1:1 ratio

3. *Tests of swollen and wild-type sisters from* 2.

Swollen female	*Wild-type (heterozygous) female*
(by wild-type or swollen male)	(by swollen male)
Only male progenies.	Only female progenies.
Swollen males.	Wild-type and swollen, 1:1 ratio

FIGURE 5.—Inheritance of "swollen" in *S. coprophila.*

the resulting progenies include wild-type and swollen in a 1:1 ratio as expected, save for a deficiency of swollen females due to poor viability. When the females of this generation are tested the swollen ones all prove to be male-producers and give exclusively swollen sons, aside from the occasional "exceptional" daughters. Conversely, their wild-type sisters are all female-producers and, if crossed to swollen males, give the same results as their mothers. This process may be repeated over and over. Judging from present evidence it will continue indefinitely, the swollen females regularly being male-producers and their heterozygous sisters female-producers.

The initial experiment involving swollen (made by Mrs. ULLIAN) was of the type mentioned above, but in the immediately succeeding experiments

the association between swollen and "sex of progeny" was not detected, owing to poor food conditions and the use of mass cultures. The writers' experiments with this character began with a cross of swollen and heterozygous females (singly) to males from Riverside, California stock. The males were the sons of wild females. These matings are summarized in table 1.

The females used here were taken from a mass culture and were selected because they all had the wing veins modified to some extent and were therefore thought to be swollen (the characteristics of swollen not being well known at that time). The second column of table 1 gives the original notes on these females. It will be observed that most of those giving male progenies were definitely recorded as swollen, whereas the others were mostly doubtful. Three of the latter also gave exceptional wild-type sons, in contrast to the sons of the male-producers.

TABLE 1

Original pair matings of virgin swollen or heterozygous females to wild-type California males.

CULTURE NUMBER*	NOTES ON PARENT ♀♀	OFFSPRING			
		♀		♂	
		+	*s*	+	*s*
2843	Very slight *s*	56	0	0	0
2853– 1	Slight *s*, like spot	58	0	0	0
2853– 2	Slight *s*, like spot	29	0	0	0
2854	*s* fairly extreme	24	0	0	0
2855– 1	Extreme *s*	0	0	0	59
2855– 2	Extreme *s*	2	0	0	83
2856– 1	Slight *s*, like spot	96	0	1	0
2856– 2	Slight *s*, like spot	67	0	1	0
2858– 1	Slight *s*, like spot	48	0	0	0
2858– 2	Slight *s*, like spot	75	0	0	0
2859– 1	Moderate *s*	12	0	0	0
2859– 2	Moderate *s*	43	0	0	0
2859– 3	Moderate *s*	75	0	2	0
2859– 4	Moderate *s*	10	0	0	0
2872– 7	*s*	3	0	0	93
2872– 8	*s* some extreme	4	0	0	58
2872– 9	*s* some extreme	23	0	0	0
2872–14	*s* some extreme	30	0	0	0
2973	*s*	0	0	0	25
2983	*s*	0	0	0	23
2984	*s*	0	0	0	27

* In this and other tables it will be noted that several similar matings are often recorded under one main number, as, for example 2859–1, 2859–2, etc. This is done purely for convenience. Each represents a separate mating.

TABLE 2

Tests of virgin females from progenies shown in table 1.†*

MATING	CULTURE NUMBER	OFFSPRING ♀ +	OFFSPRING ♀ s	OFFSPRING ♂ +	OFFSPRING ♂ s	TOTAL PROGENIES ♀:♂
♀ from 2843 by *s* ♂	2951– 1	100	0	3	1	6:7
	2951– 2	0	0	25	26	
	2965– 1	0	0	10	15	
	2965– 2	0	0	35	38	
	2965– 3	42	0	2	1	
	2965– 4	0	0	1	5	
	2965– 5	0	0	17	29	
	2965– 6	52	0	2	1	
	2965– 7	0	0	9	12	
	2965– 8	36	0	0	1	
	2965– 9	0	0	16	27	
	2965–10	15	0	0	0	
	3019	54	0	3	1	
♀ from 2853–1 by +♂	2954	43	0	3	0	
♀ from 2853–2 by +♂	2953– 1	2	0	110	64	0:2
	2953– 2	0	0	63	15	
♀ from 2854 by +♂	2962– 1	11	0	0	0	3:0
	2962– 2	95	0	0	0	
	2962– 3	95	0	0	0	
"Exceptional" ♀ from 2855–2 by *s* ♂	2985	0	0	19	20	0:1
"Exceptional" ♀ from 2872–7 by *s* ♂	2990– 1	1	0	22	25	0:3
	2990– 2	0	0	24	31	
	2990– 3	0	0	3	4	
"Exceptional" ♀ from 2872–8 by *s* ♂	2989– 1	0	0	80	80	0:3
	2989– 2	0	0	53	48	
	2989– 4	0	0	51	42	
♀ from 2872–9 by *s* ♂	2980– 1	0	0	19	33	1:4
	2980– 3	0	0	41	44	
	2980– 4	0	0	16	24	
	2980– 5	84	1	0	2	
	2980– 6	0	0	5	14	

* Seven of these are "exceptional" females, hence male-producers (series 2985, 2989 and 2990).

† The source of the mother and the genetic constitution of the father are given in column one of the table.

In the light of recent evidence it is clear that the male-producing mothers here were swollen (X*s*X*s*) and the female-producing mothers heterozygous for swollen (X′X*s*). The latter had modified wing veins due to the presence of "spot," an irregular autosomal character which is apparently exaggerated in the presence of the gene for swollen.

On such a basis it would be expected that only half the females from this cross would carry swollen. The complete records are given in table 2. Since the first thirteen of these involve sisters from one culture (2843) mated singly to swollen males they may be considered separately. Seven of the sisters proved to be male-producers, all heterozygous for swollen. The remaining six were female-producers. One of the latter gave only wild-type daughters (2965–10). The other five, however, gave, in addition to wild-type daughters, one or more "exceptional" sons, at least one of which, in each case, was classified as swollen. Some of these sons were extreme swollen and were unquestionably classified correctly. Their presence was largely responsible for the earlier conclusion that the mothers here were all heterozygous for swollen (METZ and ULLIAN 1929).

Tests of females from other progenies noted in table 1 are likewise included in table 2. Of the eighteen involved here, seven were "exceptional" individuals (2985, 2989, 2990) and hence male-producers (compare METZ and MOSES 1928). Six others were also male-producers, and, as in the other cases, all gave both wild-type and swollen, usually in a ratio of approximately 1:1. Of the five female-producers, four were mated to wild-type males and, as expected, gave only wild-type offspring. The other (2980–5) was mated to swollen and gave, in addition to wild-type daughters, one extreme swollen daughter and two swollen sons. These latter present the same difficulty as the swollen "exceptional" males mentioned in the preceding paragraph (see below).

In the next generation, when females from progenies in table 2 were tested, it was found that those from swollen fathers were heterozygous for swollen and that when mated to swollen males the female-producers gave the same type of results as the male-producers. This is shown in table 3. The swollen female class is deficient in numbers due to poor viability.

When the females from this generation were tested (table 4) it was found that the swollen ones were male-producers (X*s*X*s*) while their heterozygous sisters were all female-producers (X′X*s*). The latter were mated to swollen males and gave the same results as their mothers. Owing to difficulties with food the counts were small here in most cases.

In both of the generations just considered the results agree with the interpretation outlined in the Introduction. It remains, therefore, to account

for the results obtained in the second generation (table 2) as noted above. On the present interpretation no swollen offspring would be expected from the female-producing females in that generation. The few obtained are presumably due to non-disjunction or to other chromosomal disturbances (which appear to be unusually frequent in this material). Although a few

TABLE 3

*Tests of females from progenies given in table 2.**

MATING	CULTURE NUMBER	OFFSPRING ♀ +	OFFSPRING ♀ s	OFFSPRING ♂ +	OFFSPRING ♂ s	TOTAL PROGENIES ♀ : ♂
♀ from 2965–6 by *s* ♂	3030	36	12	0	0	2:0
	3031	17	9	0	0	
♀ from 2965–10 by *s* ♂	3020– 1	0	0	56	73	2:5
	3020– 2	0	0	55	40	
	3020– 3	0	0	74	58	
	3026– 1	0	0	25	1	
	3026– 2	7	5	0	0	
	3026– 3	0	0	43	40	
	3078	79	39	0	2	
♀ from 3019 by *s* ♂	3024	48	27	0	1	11:6
	3025– 1	Many	Many	0	0	
	3025– 2	30	27	0	0	
	3025– 3	0	0	30	28	
	3025– 4	55	17	0	0	
	3025– 5	0	0	59	51	
	3025– 6	29	14	0	0	
	3027	0	0	14	21	
	3037– 1	25	10	0	0	
	3037– 2	0	0	18	10	
	3037– 3	0	0	0	18	
	3037– 4	0	0	15	23	
	3037– 5	25	8	0	0	
	3037– 7	31	19	0	0	
	3037– 8	18	8	0	0	
	3037–10	9	2	0	1	
	3037–11	24	21	0	0	

* The source of the mother and the genetic constitution of the father are given in column one of the table.

might be due to contamination or errors in recording, this is unlikely and, in any event, such an assumption could not account for all the cases. The one extreme swollen female, for example, was particularly noted and could

not have been due to contamination because no other swollen females were in the laboratory at the time. This female gave no offspring, apparently being sterile. It is possible that she was a mosaic in which the wings were male and exhibited swollen, although the fly was genetically female and

TABLE 4

Tests of females from table 3.
A. swollen females Xs/Xs. B. heterozygous females X'/Xs.

MATING	CULTURE NUMBER	OFFSPRING ♀ +	OFFSPRING ♀ s	OFFSPRING ♂ +	OFFSPRING ♂ s	TOTAL PROGENIES ♀ : ♂
A.						
♀ from 3025–6 by + or *s* ♂	3093 (mass)	0	0	0	14	0:1
♀ from 3030–1 by + or *s* ♂	3090– 1	0	0	0	40	0:2
	3090– 2	0	0	0	88	
♀ from 3078 by + or *s* ♂	3082– 1	0	0	0	101	0:1
B.						
	3085– 3	12	4	0	2	
	3085– 5	8	2	0	1	
	3085– 6	24	11	0	0	
	3085– 8	7	5	1	3	
	3085–10	21	14	0	0	
♀ from 3024 by *s* ♂	3085–15	13	8	0	0	11:0
	3088– 4	9	7	0	0	
	3088– 5	21	15	2	0	
	3088– 8	15	13	0	1	
	3088–12	17	3	1	0	
	3088–13	10	3	0	0	
	3079– 1	Many	Many	0	0	
	3079– 2	Many	Many	0	0	
♀ from 3078 by *s* ♂	3079– 3	Many	Many	0	0	6:0
	3079– 4	36	20	0	1	
	3079– 5	Many	Many	0	0	
	3143	51	15	0	0	

heterozygous. Individuals of this type have been obtained in subsequent experiments. It should be noted that exceptional swollen males were only obtained from matings in which the father was swollen. Six of the seven matings of this type gave such males, whereas the four in which the father

TABLE 5

Test of swollen and heterozygous sisters in successive generations.

A. SWOLLEN $\frac{X_s}{X_s}$							B. HETEROZYGOUS $\frac{X'}{X_s}$						
Source of ♀	Parent ♂	Offspring ♀		Offspring ♂		Culture Number	Source of ♀	Parent ♂	Offspring ♀		Offspring ♂		Culture Number
		+	s	+	s				+	s	+	s	
3085– 5	+	1	0	0	30	3160– 2	3085– 5	+	18	0	0	0	3156–1
							3085– 5	+	79	0	0	0	3156–2
							3085– 5	+	17	0	0	0	3156–3
							3085– 5	s	16	13	0	0	3162
3085–10	+	0	0	0	12	3164– 1	3085–10	s	39	26	0	0	3163–1
3085–10	+	0	0	0	35	3223– 1	3085–10	s	45	24	0	0	3163–2
3085–10	+	0	0	0	33	3223– 2	3085–10	s	22	5	0	0	3163–3
3085–10	+	0	0	0	74	3223– 5							
3085–10	+	0	0	0	14	3223– 7							
3085–10	+	0	0	0	30	3223–10							
3085–11	+	0	0	0	14	3172– 2	3085–11	s	14	5	1	0	3174–2
3085–11	+	0	0	0	26	3173– 1							
3085–11	+	3	0	0	23	3173– 2							
3143	s	0	0	0	13	3144	3143	s	32	54	0	0	3145–1
3143	s	0	0	0	29	3148– 1	3143	s	39	17	0	0	3145–6
3143	s	0	0	2?	23	3148– 2	3143	s	33	8	0	0	3145–7
3143	s	0	0	0	14	3148– 4	3143	?	16	11	0	0	3149
3143	s	0	0	0	86	3148– 6							
3143	s	0	0	0	29	3148– 7							
3143	s	0	0	0	16	3148– 8							
3143	s	0	0	2?	19	3148– 9							
3143	s	0	0	0	41	3148–10							
3143	s	0	0	0	52	3148–11							
3143	s	0	0	0	44	3148–12							
3186– 2	+	11	0	0	41	3260– 1	3186– 2	s	19	22	0	0	3259–2
3186– 2	+	0	0	0	76	3260– 4	3186– 2	s	16	0	0	0	3259–5
3186– 2	+	0	0	1	71	3260– 5							
3186– 2	+	9	0	0	70	3260– 7							
3197–10	+	1	0	0	102	3274– 1	3197–10	s	53	40	0	0	3275–1
3197–10	+	0	0	0	102	3274– 2	3197–10	s	27	10	0	0	3275–2
3197–10	+	0	0	0	12	3274– 3	3197–10	s	18	19	0	0	3275–3
3197–10	+	1	0	0	75	3274– 4	3197–10	s	28	29	0	0	3275–4
3197–10	+	0	0	0	59	3274– 5							
3197–10	+	0	0	0	20	3274– 7							
3197–10	+	0	0	0	97	3274– 8							
3197–10	+	0	0	0	97	3274– 9							

TABLE 5 (*continued*)

A. SWOLLEN X^s / X_s							B. HETEROZYGOUS X^s / X_s						
Source of ♀	Parent ♂	Offspring ♀ +	Offspring ♀ s	Offspring ♂ +	Offspring ♂ s	Culture Number	Source of ♀	Parent ♂	Offspring ♀ +	Offspring ♀ s	Offspring ♂ +	Offspring ♂ s	Culture Number
3388– 1	+	0	0	0	57	3467– 4	3388– 1	s	30	6	0	0	3468–1
3388– 1	+	0	0	0	131	3467– 5	3388– 1	s	38	29	1	0	3468–2
3388– 1	+	0	0	0	17	3467– 6	3388– 1	s	77	65	0	1	3468–3
							3388– 1	s	47	48	0	1	3468–4
							3388– 1	s	26	28	0	0	3468–5
3847– 5	+	2	0	0	70	4063– 1	3847– 5	s	20	10	0	0	4066–2
3847– 5	+	0	0	0	7	4063– 2	3847– 5	s	22	19	0	0	4066–3
3847– 5	+	9	0	0	19	4063– 3	3847– 5	s	50	34	0	0	4066–4
3847– 5	+	11*	0	0	20	4063– 4							
3847– 5	+	0	0	0	43	4063– 5							
3847– 5	+	0	0	0	29	4063– 7							
3847– 5	+	49*	0	0	25	4063– 8							

* The females in these two progenies, although unusually numerous, are apparently all true "exceptional" individuals. Three of the eleven and fourteen of the forty-nine were tested and proved to be male-producers.

was wild-type gave none. Similar cases in other experiments make it probable that such males get their X-chromosome from the father.

Soon after the above results were obtained another cross was made between swollen and the California strain, to serve as a check on the earlier experiments. In this cross a "digenic" strain (giving bisexual progenies) was used, and the cross was the reciprocal of that made first. This digenic strain had arisen from the California stock used in the other case. Females from this were mated to swollen males and the daughters backcrossed to swollen. These daughters all should have been heterozygous for swollen, and since they gave bisexual progenies it was thought that the results would serve as a critical check on those previously secured. The counts from the backcross showed an almost complete absence of swollen females. Such a result seemed to confirm the earlier, erroneous, conclusion that the absence of swollen females following the supposed backcross was due to some genetic peculiarity of the California race (see METZ 1929, pp. 491–492). It was only after numerous experiments with both strains that the two sets of results were found to be due to different causes.

TABLE 6

Descendants from a heterozygous female-producing female X′/Xs by wild male (see text for explanation).

MATING	CULTURE NUMBER	OFFSPRING ♀ +	OFFSPRING ♀ s	OFFSPRING ♂ +	OFFSPRING ♂ s	TOTAL PROGENIES ♀:♂
♀ from 3340–1 by + ♂	3429– 1	0	0	58*		6:5
	3429– 2	0	0	101		
	3429– 3	69	0	0	0	
	3429– 4	0	0	61		
	3429– 5	0	0	31	34	
	3429– 6	139	0	0	0	
	3429– 7	0	0	28	54	
	3429– 8	66	0	1?	0	
	3429– 9	122	0	2	0	
	3429–10	162	0	0	0	
	3429–11	146	0	0	0	
♀ from 3340–1 by s ♂	3434– 1	144	0	1	0	2:2
	3434– 2	116	0	3	0	
	3434– 3	0	0	85	78	
	3434– 4	1	0	70	73	
♀ from 3429–3 by s ♂	3565– 1	0	0	12	0	2:4
	3565– 3	0	0	93	0	
	3565– 4	134	0	1	0	
	3565– 5	188	0	0	0	
	3565– 6	1	0	132	0	
	3565– 7	4	0	23	0	
♀ from 3429–6 by + ♂	3543– 1	154	0	0	0	2:2
	3543– 2	0	0	110	0	
	3543– 3	0	0	14	0	
	3543– 4	147	0	0	0	
♀ from 3429–8 by s ♂	3566– 1	161	0	0	0	5:1
	3566– 2	174	0	1	0	
	3566– 4	68	0	0	0	
	3566– 5	0	0	69	0	
	3566– 6	139	0	0	0	
	3566– 7	91	0	3	0	
♀ from 3429–9 by s ♂	3576– 2	48	0	0	0	3:1
	3576– 3	0	0	123	0	
	3576– 4	170	0	2	0	
	3576– 5	159	0	0	0	
♀ from 3429–11 by s ♂	3567– 2	181	0	0	0	3:2
	3567– 4	0	0	141	0	
	3567– 5	0	0	183	0	
	3567– 6	96	0	0	1	
	3567– 7	22	0	0	0	

*Records in the central column are from progenies in which the two classes of flies were present but were not counted separately.

The behavior of the "digenic" strain will be considered in another paper. It is distinctly different from that of the "monogenic" strains,[5] due apparently to some chromosome disturbance.

In the monogenic strains the inheritance of swollen has been followed for more than ten generations, with regular conformity to the scheme outlined above. The experiments have involved various out-crosses as well as a continuation of tests of swollen and heterozygous sisters in successive generations following that shown in table 4. The latter data are too extensive to be given fully in the tables. For this reason only a few have been included from any one generation. These are given in table 5.

One of the out-crosses just mentioned was a repetition of the original cross considered in this paper (table 1), that is, a cross between a female-producing female heterozygous for swollen (X′X*s*) and a wild-type male (XY) from Riverside, California stock (Line 2860). The F_1 consisted of 92 wild-type females and no males (No. 3340). Half of these females should be homozygous wild-type and half heterozygous for swollen, and on our hypothesis the former should all be female-producers (X′X) and the latter all male-producers (XX*s*).

Eleven of the females were mated singly to wild-type males, also from the Riverside stock, with the results shown in the first part of table 6 (3429 1–11). Six gave female progenies and five male progenies. The former progenies consisted entirely of wild-type flies, the latter all included both wild-type and swollen in large numbers, showing that the male-producing mothers were all heterozygous for swollen. At the same time four F_1 females were backcrossed to swollen males, with the same type of results, as shown in table 6 (3434 1–4). The two male progenies included both wild-type and swollen.

These results agree fully with expectation, the F_1 male-producers all being heterozygous for swollen, and the female-producers pure wild-type so far as tested. In the 3429 series the latter feature is uncertain since the fathers were wild-type. On our hypothesis none of the daughters in this series (3429 1–11) should carry swollen. To test this 29 were mated to swollen males. Twenty-five gave offspring, as shown in the latter part of table 6 (series 3543, 3565, 3566, 3567, 3576). Both the male and the female progenies were wild-type, as expected. The one exceptional swollen male in 3567–6 is evidently like those considered above—due presumably to non-disjunction.

[5] This difference, however, is not such as to cast doubt on any essential features in the present paper.

Additional data on the inheritance of swollen are given in the experiments dealing with narrow.

INHERITANCE OF NARROW

The mode of inheritance of narrow is the same as that of swollen, although the percentage of narrow flies is usually considerably reduced by poor viability, which in this case affects males as well as females. Owing to the fact that narrow first appeared in a digenic strain, as noted above, the earlier experiments with this character will be considered in another paper. Those treated here are from matings of narrow males to monogenic females, either pure wild-type or heterozygous for swollen.

In one experiment (3930) a female-producing female from wild stock 3059 (pure Anaheim, California stock) was mated to narrow males.[6] The F_1 consisted of approximately 125 wild-type females, no males. Four of these females were backcrossed to narrow males (mass culture 4116). Presumably only one gave offspring, for the progeny consisted of 12 wild-type and 15 narrow flies, all females. The former should all be female-producers ($X'Xn$) and the latter all male-producers ($XnXn$). Nine of the former were tested by mating to wild-type males (all but two from Anaheim stock 3059)

TABLE 7

Tests of narrow and heterozygous sisters (see text).

A. NARROW ♀ $\frac{Xn}{Xn}$ BY + ♂						B. HETEROZYGOUS ♀ $\frac{X'}{Xn}$ BY + ♂					
Source of ♀	Culture Number	Offspring				Source of ♀	Culture Number	Offspring			
		♀		♂				♀		♂	
		+	*n*	+	*n*			+	*n*	+	*n*
4116	4256–1	0	0	0	52	4116	4255–1	198	0	1	1
4116	4256–2	0	0	0	65	4116	4255–2	183	0	0	2
4116	4256–3	0	0	0	69	4116	4265–1	200±	0	0	0
4116	4280–2	0	0	0	34	4116	4265–3	60±	0	0	0
4116	4280–4	0	0	0	8	4116	4265–5	50±	0	0	0
4116	4293–1	0	0	0	46	4116	4265–6	150±	0	2	0
						4116	4265–7	100±	0	0	0
						4116	4265–8	125±	0	0	0
						4116	4279	150±	0	0	0

[6] Owing to the poor viability of narrow males it has been customary to use several in each mating rather than try to mate them singly. Presumably the female is in each case fertilized by only one male. (Compare MOSES and METZ 1928.)

and six of the latter by mating to wild-type males, also partly from Anaheim stock. The results conform to expectation as shown in table 7.

The next experiment was of a similar nature but more extensive. It is summarized in tables 8 to 10. Here narrow males were mated to a wild-type female from pure San Juan Capistrano, California stock, number 3086. This mating (3932) gave approximately 100 wild-type females, no males. Fourteen of these females were tested singly by mating to swollen males as shown in table 8A (4108 series). At the same time three mass matings were made of three females each to narrow males, with the result shown in table 8B (4136 series).

TABLE 8

Tests of F_1 female from pure wild-type ♀ by narrow ♂. A. F_1 ♀ by s ♂. B. F_1 ♀♀ by n ♂♂ (mass matings).

	SOURCE OF ♀	CULTURE NUMBER	OFFSPRING ♀ +	♀ n	♂ +	♂ n
A.	3932	4108– 1	120	0	0	0
	3932	4108– 2	0	0	60	63
	3932	4108– 3	0	0	80	52
	3932	4108– 4	147	0	0	0
	3932	4108– 5	0	0	88	69
	3932	4108– 6	147	0	0	0
	3932	4108– 7	0	0	86	47
	3932	4108– 8	167	0	0	0
	3932	4108– 9	0	0	39	31
	3932	4108–10	0	0	47	62
	3932	4108–11	0	0	55	64
	3932	4108–12	0	0	85	64
	3932	4108–14	97	0	0	0
	3932	4108–15	0	0	52	61
					592	513
B.	3932	4136– 1	70	51	0	0
	3932	4136– 2	0	0	64	33
	3932	4136– 3	36	10	39	17

From the former series the daughters were, as expected, all wild-type. Thirty-three of them were tested singly by mating to narrow males, as shown in table 9A. These should be of two kinds, half female-producers

TABLE 9

Tests of virgin females from progenies shown in table 8.

A. $\frac{X'}{Xs}$ —♀ *and* $\frac{Xn}{Xs}$ —♀ *from table 8A. B.* $\frac{X'}{Xn}$ —♀ *and* $\frac{Xn}{Xn}$ —♀ *from table 8B.**

MATING	CULTURE NUMBER	OFFSPRING ♀ +	♀ n	♂ +	♂ n	♂ s	♂ ns	TOTAL PROGENIES ♀:♂
A.								
	4267– 1	0	0	0	4	17	2?	
	4267– 2	67	0	0	0	0	1?	
♀ from 4108–4 by *n*♂	4267– 4	112	0	0	0	0	0	4:1
	4267– 5	69	0	0	0	2	0	
	4267– 8	13	0	0	0	0	0	
	4245– 2	0	0	1	43	90	0	
	4246– 1	128	0	0	0	1	0	
	4246– 2	0	0	0	56	80	0	
	4246– 3	192	0	0	0	0	0	
	4247– 1	108	0	0	0	0	0	
	4247– 2	2	0	0	30	51	0	
	4247– 3	0	0	0	12	27	0	
	4247– 4	38	0	0	0	0	0	
	4247– 5	76	0	0	0	1	0	
	4247– 6	0	0	0	20	49	2?	
	4247– 7	53	0	0	0	0	0	
	4247– 8	111	0	0	0	0	0	
	4247– 9	0	0	0	29	50	0	
♀ from 4108–6 by *n*♂	4247–10	0	0	1	67	78	0	15:13
	4247–11	138	0	0	0	0	0	
	4285– 5	101	0	0	0	0	0	
	4285– 6	0	0	0	7	25	2?	
	4285– 7	83	0	0	0	0	0	
	4285– 8	0	0	0	8	26	0	
	4285–10	0	0	0	16	24	10?	
	4285–11	0	0	0	14	26	2?	
	4285–12	70	0	0	0	0	0	
	4285–13	0	0	0	17	33	0	
	4285–14	78	0	0	0	0	0	
	4285–16	84	0	0	0	1	0	
	4289– 2	0	0	0	39	63	3?	
	4289– 4	35	0	0	0	0	0	
	4292	50±	0	0	0	0	0	
B.								
	4264	80	38	0	0	0	0	
+ ♀ from 4136–1 by *n*♂	4281	6	2	0	1	0	0	3:0
	4291	18	13	0	0	0	0	

TABLE 9 (*continued*)

MATING	CULTURE NUMBER	OFFSPRING ♀ +	♀ *n*	♂ +	♂ *n*	♂ *s*	♂ *ns*	TOTAL PROGENIES ♀:♂
	4295–1	125±	0	0	0	0	0	
	4295–2	100±	0	0	0	0	0	
	4295–3	28	0	0	0	0	0	
+♀ from 4136–1 by +♂	4295–4	50±	0	0	0	0	0	7:0
	4295–5	100±	0	0	0	0	0	
	4295–6	50±	0	0	0	0	0	
	4295–7	150±	0	0	0	0	0	
+♀ from 4136–1 by *s*♂	4352	50±	0	0	0	0	0	
	4317–1	0	0	0	10	0	0	
n♀ from 4136–1 by ?♂	4317–2	0	0	0	10	0	0	0:3
	4317–3	0	0	0	13	0	0	
n♀ from 4136–1 by +♂	4294–1	0	0	0	40	0	0	0:2
	4294–2	0	0	0	54	0	0	
	4269	100±	0	0	0	0	0	
	4299–1	30	0	0	0	0	0	
	4299–2	200±	0	0	0	0	0	
	4299–3	200±	0	0	0	0	0	
	4299–4	200±	0	0	0	0	0	
+♀ from 4136–3 by *n* and	4299–5	100±	0	0	0	0	0	
+♂♂	4315–1	43	0	0	0	0	0	15:0
	4315–2	89	0	0	0	0	0	
	4315–3	18	0	4	0	0	0	
	4315–4	105	0	0	0	0	0	
	4315–5	92	0	0	0	0	0	
	4315–6	128	0	0	0	0	0	
	4315–7	30	0	0	0	0	0	
	4315–8	100	0	0	0	0	0	
	4315–9	125±	0	0	0	0	0	
+♀ from 4136–3 by +♂	4282–1	75	0	0	0	0	0	
	4282–2	86	0	0	0	0	0	2:0
	4313–3	19	0	0	30	0	0	
n♀ from 4136–3 by *n* and	4313–4	0	0	0	12	0	0	
+♂♂	4313–5	0	0	0	24	0	0	0:4
	4313–6	0	0	0	55	0	0	

* The source of the mother and the genetic constitution of the father are given in column one of the table.

heterozygous for swollen but not for narrow (X′X*s*) and half male-producers heterozygous for both swollen and narrow (X*s*X*n*). If any of the former carried narrow they should give some narrow daughters, and if any of the latter failed to carry either narrow or swollen the corresponding class of sons should be absent. The results are: 19 female progenies, all wild-type aside from the few expected "exceptional" swollen sons; and 14 male progenies, all of which consist of narrow and swollen males together with a few "crossover" individuals. Those classified as "*n s* ?" are probably all merely swollen, not narrow (see below under linkage).

From the second series of matings of F_1 females (4136 series in table 8B) both wild-type and narrow daughters were obtained, as expected. The former should be female-producers and the latter male-producers. Thirty-seven were tested, with the expected results, as shown in table 9B. Most of the matings were to wild-type males. In the three cases where female progenies were sired by narrow males both wild-type and narrow daughters were obtained, as expected. In the case of culture 4313–3 where 19 females and 30 males were secured it is possible that two mothers were accidentally put together, although the females probably were true "exceptional" individuals. Seven were mated to test this feature, but only one gave offspring. She gave wild-type and narrow males as would be expected if she were "exceptional."

From two of the progenies just considered, in which both wild-type and narrow females were secured (4264 and 4281) a few additional matings were made. The narrow females gave no offspring. The wild-type matings are summarized in table 10. Five of these are pair matings and one (4476) a mass mating, in which, judging by the large count, at least two females

TABLE 10

Tests of heterozygous sisters of narrow females (see text).

MATING	CULTURE NUMBER	OFFSPRING ♀ +	♀ *n*	OFFSPRING ♂ +	♂ *n*	TOTAL PROGENIES ♀:♂
♀ from 4264 by *s* ♂	4459–1	50±	0	0	0	3:0
	4459–2	50±	0	0	0	
	4468–2	50±	0	0	0	
♀ (mass) from 4264 by *n*♂	4476	135	93	0	1	1:0
♀ from 4281 by *n*♂	4473–1	82	38	0	0	2:0
	4473–2	76	39	0	0	

gave offspring. All of the females were female-producers as expected, and in the three cases where matings were made to narrow males the offspring included both wild-type and narrow as expected.

Another series of experiments (4061 line) involved both narrow and swollen. Here a female-producing female heterozygous for swollen, from swollen "stock," was mated to a narrow male (or males?). The F_1 consisted of 16 wild-type females. These were mated singly to narrow or swollen males, with the results shown in table 11, which is divided into two parts to separate female from male progenies. The type of parent male used in each mating is shown in the second column. As expected, the females proved to be of two types, half female-producers heterozygous for narrow,[7] and half male-producers heterozygous for both narrow and swollen.

TABLE 11

Tests of daughters from heterozygous female $\frac{X'}{Xs}$ *by narrow male* $\frac{Xn}{Y}$ *(4061 line).*

A. ♀—PROGENIES							B. ♂—PROGENIES								
Source of ♀	Parent ♂	Offspring ♀		Offspring ♂		Culture Number	Source of ♀	Parent ♂	Offspring ♀			Offspring ♂				Culture Number
		+	*n*	+	*n*				+	*n*	*s*	+	*n*	*s*	*ns*	
4061	*n*	64	14	0	0	4231– 2	4061	*n*	1	1	0	0	21	53	0	4231–1
4061	*n*	55	39	1	1	4231– 4	4061	*n*	1	0	0	2	3	24	0	4231–3
4061	*n*	46	16	0	0	4237– 2	4061	*n*	2	0	0	0	26	55	0	4237–3
4061	*n*	38	10	0	0	4237– 5	4061	*n*	0	0	0	0	8	14	0	4237–4
4061	*n*	60	19	0	0	4237– 6										
4061	*s*	64	0	0	0	4239– 7	4061	*s*	0	1	0	1	32	45	0	4239–1
4061	*s*	96	0	0	0	4239– 9	4061	*s*	1	0	0	1	5	47	0	4239–2
4061	*s*	66	0	0	0	4239–10	4061	*s*	0	0	0	0	18	27	0	4239–3
							4061	*s*	2	0	3	0	37	65	2	4239–8

From the female progenies wild-type and narrow sisters were tested. The former should be female-producers and be heterozygous for narrow (X′X*n*). They were, therefore, mated to narrow males, with one exception. As shown in table 12 they proved to be of the expected constitution. Only two of the narrow females gave offspring. One gave four and the other thirteen sons, all narrow as expected.

[7] Three of these were mated to swollen males and consequently did not reveal their heterozygous constitution.

TABLE 12

Tests of $\frac{X'}{Xn}$ females (4061 line).

SOURCE OF ♀	PARENT ♂	OFFSPRING ♀ +	OFFSPRING ♀ n	OFFSPRING ♂ +	OFFSPRING ♂ n	CULTURE NUMBER
4231–2	+	36	0	0	0	4451
4237–5	n	37	18	0	0	4407
4237–5	n	30	13	0	0	4421–1
4237–5	n	84	33	0	0	4421–2
4237–5	n	22	6	0	0	4421–3
4237–5	n	68	28	0	0	4421–5
4237–5	n	63	40	0	1	4421–6
4237–5	n	19	7	0	0	4421–7
4237–6	n	47	26	0	1	4423–2
4237–6	n	99	63	0	0	4423–3
4237–6	n	94	58	0	1	4423–4

LINKAGE OF SWOLLEN AND NARROW

It will be seen from the data presented above (tables 9A and 11B) that narrow and swollen are closely linked. To supplement these records a few additional counts are given in table 13. For present purposes the exact amount of crossing over between the two is not especially important. The significant fact is that crossing over does occur, and with sufficient frequency to make it readily detected. The evidence for this conclusion will be

TABLE 13

Tests of $\frac{Xn}{Xs}$ females by narrow and swollen males.

CULTURE NUMBER	OFFSPRING ♀ +	OFFSPRING ♀ n	OFFSPRING ♂ +	OFFSPRING ♂ n	OFFSPRING ♂ s	OFFSPRING ♂ ns
4437	0	0	4	20	42	1
4444–1	0	0	1	14	35	7?
4472–3	0	0	0	32	42	0

considered in detail because of its bearing on the features considered in the following section. Since the crossover classes are both small, and since irregularities in chromosome behavior are evidently frequent in this material, it is particularly necessary to make sure that the flies in these classes

are not due to such irregularities. As noted in considering the inheritance of swollen alone, a male appears occasionally which evidently received his X-chromosome from his father. This casts some doubt on the origin of wild-type males wherever the father is wild-type. Consequently in the matings under consideration only narrow or swollen males have been used. It seems certain, therefore, that the flies recorded in the wild-type crossover class are actually due to crossing over. The only other obvious possibilities are contamination, reverse mutation or mistakes in classification. Since the characters swollen and narrow are both readily distinguished from wild-type in males, and since particular attention was given to the classification here, the latter possibility may be ruled out. That contamination is not responsible seems certain for three reasons (1) because of the methods used, (2) because similar results are not found in other experiments and (3) particularly because of the presence of the reciprocal crossover class, which cannot be due to contamination. The last two reasons apply with equal force to the possibility of reverse mutation and, in our opinion, effectively eliminate this as a causal factor here.

In considering the narrow swollen crossover class it is to be observed first that the double recessive males here (females of this sort are, of course, not obtained) have very poor viability and have all failed to breed. As a consequence it has not been possible to make the reciprocal type of matings involving narrow and swollen in the same chromosome. Likewise in considering the narrow swollen crossover class, it will be observed that most of the records in this class are followed by a query (?). Flies thus designated were swollen, but had their wings incompletely expanded or otherwise affected in such a way as to resemble narrow or to prevent the detection of narrow. A few of them may have been narrow, but most of them must have been merely swollen. On the other hand a few unquestionable narrow swollen flies were secured and most, if not all, cases so recorded are of this type.

Taking the tables as they stand, and omitting the few questionable individuals just considered, we find a total of 1486 non-crossover to 8 crossover individuals, or a crossover frequency of approximately 0.5 percent. This value is probably too small, due to the extreme inviability of narrow swollen flies. A better calculation may be that based on only the wild-type and swollen classes, which gives 0.62 percent.

SEX LINKED INHERITANCE OF "SEX OF PROGENY"

It is evident throughout the experiments considered in this paper that "sex of progeny" is inherited as a distinct character, that it is sex linked,

and that it is determined by the sex chromosome constitution of the mother, irrespective of the male parent. It is also seen that the difference between the two types of mothers in this respect is a definite and constant one. The two sex chromosomes of the male-producing female are essentially alike in constitution and origin and are interchangeable. One is derived directly from the father and the other indirectly from the maternal grandfather. Those of the female-producing female, on the other hand, are unlike in both make-up and derivation and are not interchangeable. One comes from the father and is like those in the male-producer; the other comes from the mother, grandmother, etc., in a direct female line of descent, without having passed through either a male-producing female or a male (ordinarily).[8] The difference between this latter chromosome (X′) and the ordinary X, represents at least the differential responsible for determining sex of progeny, in accordance with the scheme shown in figure 1.

POSSIBLE ABSENCE OF CROSSING OVER BETWEEN X′ AND X

At the present time it is too early to attempt a detailed comparison between X′ and X. We know that these two chromosomes differ in respect to the differential responsible for sex of progeny. But in the absence of crossing over between the two (see below) we do not know whether this differential is a single gene or a complex of genes; nor do we know whether the difference in this respect constitutes the sole difference between the two chromosomes. On the other hand, several significant lines of evidence have been obtained which bear on the relation between these chromosomes and serve at least to limit the possibilities. These are summarized below in two categories—those indicating resemblances and those indicating differences. Particular consideration is given to the crossing over relationships.

Indications of similarity

It has been possible, thus far, to identify five combinations of sex chromosomes in this material, namely, XX, X′X, X′X′, XY and X′Y.[9] The first three of these produce females and the last two males. The three kinds of females appear to be indistinguishable somatically. Similarly the two kinds of males look alike, at least in general morphological features. All five types are viable and fertile. It appears, therefore, that X′ can be substituted for X in any combination without noticeable effect on the external characteristics or viability of the fly. From this we infer that X and X′ are

[8] See discussion of exceptional X′ males in next section.

[9] The combinations X′Y and X′X′ are derived rarely, as considered elsewhere (Metz and Schmuck 1929).

fundamentally alike and that their genic constitutions are much more nearly the same than, for example, those of X and Y.

Further support for this view is given by the evidence presented above showing that X′ carries the normal allelomorphs of the two mutant genes thus far found in X.

Indications of dissimilarity

Contrasted with the above, and possibly equally significant, is the fact that in using the two mutant genes just mentioned we have been unable to detect any crossing over between X and X′, and have, in consequence, been unable to get either gene transferred from X to X′. The weight of this evidence is, of course, influenced by the fact that the two genes (swollen and narrow) are closely linked and show only a small amount of crossing over in XX females.

If we assume that sex of progeny is "determined" by a single gene (pair) we may consider the linkage relations between this gene and the genes for swollen and narrow. Should the locus of the former not lie between the loci of swollen and narrow on the chromosome then it should cross over more freely with one of the latter than these two do with one another—providing the rate of crossing over between X and X′ were the same as between X and X. On the other hand, if the gene for sex of progeny lay between the loci of swollen and narrow the reverse should be true. In this case, however, the frequency of crossing over with one of the latter two genes should be at least half that between the two (swollen and narrow) alone. Such a frequency (probably not over 0.5 percent) would obviously be difficult to detect in the present case.

However, it is to be observed that in addition to using swollen and narrow in pair matings as noted above, both characters have been used in mass matings on a large scale. These latter have served both as experiments and for the purpose of keeping stock. Where swollen is involved, for example, each culture received swollen males, swollen male-producing females and heterozygous female-producing females. Thus all the X chromosomes carry swollen, and as long as swollen does not cross over into the X′ chromosome the stock is self perpetuating. If such crossing over should occur, however, it would be manifest by the presence of wild-type males or swollen female-producing females in large numbers. In the case of narrow any such crossing over would be shown in the same way.

It is difficult to estimate how many flies have been tested in this manner, but the numbers must be large, particularly in the case of swollen, which has thus been under observation for fifteen generations or more. If the

(hypothetical) gene for sex of progeny were "located" very close to that for swollen it is possible that crossing over between X′ and X might occur as frequently as between X and X without having been detected in our experiments. In any other position, however, such a frequency should have been detected.

As the evidence stands it suggests that either X and X′ differ in respect to more than one gene, or else this gene influences not only sex of progeny but also the synaptic behavior of the sex chromosomes—reducing or eliminating crossovers between X and X′. However, a study of additional mutant characters will probably be required to clear up this point, for it is possible that the general rate of crossing over in this material is low, as compared, for example, with that in Drosophila, and that genes actually far apart on the chromosome show close linkage.

At present only one other feature calls for comment. This is the widespread distribution throughout the genus Sciara of the characteristics found in the present species,[10] that is, production of unisexual progenies and presence of two types of females presumably differentiated by X and X′ chromosomes. Such evidence tends to emphasize the relative stability of the distinction between X and X′, whatever its magnitude.

DISCUSSION

The numerous genetic and cytological peculiarities in Sciara obviously invite speculation and comparison—particularly speculation as to the mode of origin and phylogenetic significance of the principal features. In our papers, however, we have endeavored to avoid considerations of this kind, so far as possible, in the belief that they would be of little value until more evidence is available. We still hold to this belief and consequently limit the present discussion to a few topics. Most of the special aspects have been discussed above under the separate headings or in the Introduction.

So far as we are aware no parallel to the processes of sex determination in Sciara has been recorded among other organisms. Unisexual progenies are well known in various groups of animals, such as Nematodes, Rotifers, Daphnids, Aphids, Phylloxerans, Coccids, etc.,[11] but in these groups the unisexual type of progeny is connected with such phenomena as parthenogenesis, gynogenesis[12] and alternation of generations, none of which is found in Sciara. A more nearly comparable case may be that recently recorded

[10] Compare METZ 1928.

[11] Conditions in these groups have been reviewed frequently and exhaustively. See especially SCHRADER 1928, WILSON 1925, SHULL 1929, GOLDSCHMIDT 1923 and BELAR 1928.

[12] See WILSON 1925 and BELAR 1928.

by BARNES (1929) in the gall midge *Rhabdophaga heterobia* H. Lw. Here unisexual families are found and a case is recorded in which sister females gave progenies of opposite sexes. It is not yet known, however, which parent is responsible for the sex of the progeny or how the unisexuality is brought about. Should it be found that the phenomena here are fundamentally like those in Sciara it would suggest that the condition is widespread among the lower Diptera.

As regards selective fertilization, any discussion or conclusions must be tentative, for it has not been possible to prove directly that such a process occurs here, and one alternative possibility has been specifically outlined (METZ 1930). If we consider the present indirect evidence conclusive, however, it is of interest to compare conditions here with those in other organisms, and also to consider their mode of origin.

Numerous cases have been described in which one type of sperm appears to be more effective than the other,[13] but in none of these, so far as we are aware, are there regularly two types of sister females exhibiting selective fertilization of opposite types and almost one hundred percent efficiency.

These two types of females, and of selective fertilization, in Sciara present a particularly interesting problem. If only one type of selective fertilization were present it would be simple to postulate the occurrence of a mutation in the X chromosome which produced the effect; but the origin of two opposite types in the same material is not so readily accounted for. It is difficult to conceive of the two arising simultaneously. It would seem more probable that they arose through a succession of steps. If so, the difference between the two types of females might well involve more than one pair of genes.

In respect to the sex chromosomes themselves, it seems probable that we are dealing with a modification of the ordinary XX–XY mechanism, although the extent of the modification cannot be determined at present. The widespread distribution of the X′ chromosome throughout the genus, as noted above, indicates that the condition has been in existence a long time. Any consideration of its origin, however, is complicated by other features, especially the presence of the large "androsomes" or "male-limited" chromosomes, whose behavior suggests that they have at some time been directly concerned with sex determination (see Addendum).

SUMMARY

See last six paragraphs of the Introduction and concluding paragraph of the Discussion.

[13] See reviews by KING 1929 and by JONES 1928.

ADDENDUM

After the manuscript of this paper was sent to press additional cytological evidence was obtained which may throw a new light on the problems discussed above. This evidence indicates that the so-called "androsomes" or "male-limited" chromosomes may be present in the *germ line* of both sexes, but not in the soma of either sex. It also indicates that in the *male* one sex chromosome is eliminated from the soma at an early stage, and that this process of elimination may be concerned with sex determination. A preliminary note on this subject is in press in the *Biologische Zentralblatt.*

LITERATURE CITED

BARNES, H. F., 1929 Unisexual families in *Rhabdophaga heterobia* H. Lw. (Cecidomyidae, Diptera). Ent. Mon. Mag. **65**: 256–257.

BELAR, KARL, 1928 Die Cytologische Grundlagen der Vererbung. Handb. d. Vererbungsw. I. 412 pp., 280 figs. Berlin: Gebrüder Borntraeger.

GOLDSCHMIDT, R., 1923 The mechanism and physiology of sex determination. 259 pp. New York: G. H. Doran.

JOHANNSEN, O. A., 1912 The fungus gnats of North America, Part IV, Maine Agric. Expt. Sta. Bull. No. **200**: 57–145.

JONES, D. F., 1928 Selective fertilization. Chicago: Univ. Chicago Press.

KING, H. D., 1929 Selective fertilization in the rat. Arch. EntwMech. Org. **116**: 202–219.

METZ, CHARLES W., 1927 Chromosome behavior and genetic behavior in Sciara (Diptera). II. Genetic evidence of selective segregation in *S. coprophila*. Z. indukt. Abstamm.-u. VererbLehre. **45**: 184–200.

1928 Data cited in Yearb. Carnegie Instn. **27**: 52.

1929 Evidence that "unisexual" progenies in Sciara are due to selective elimination of gametes (sperms). Amer. Nat. **63**: 214–228.

1929 Sex determination in Sciara. Amer. Nat. **63**: 487–496.

1930 A possible alternative to the hypothesis of selective fertilization in Sciara. Amer. Nat. **64**: 380–382.

METZ, CHARLES W., and MOSES MILDRED S., 1928 Observations on sex-ratio determination in Sciara (Diptera). Proc. Nat. Acad. Sci. Wash. **14**: 930–932.

METZ, CHARLES W., MOSES, M. S., and HOPPE, ELLAN N., 1926 Chromosome behavior and genetic behavior in Sciara (Diptera). I. Chromosome behavior in the spermatocyte divisions. Z. indukt. Abstamm.-u. VererbLehre. **42**: 237–270.

METZ, CHARLES W., and ULLIAN, SILKA S., 1929 Genetic indentification of the sex chromosomes in Sciara (Diptera). Proc. Nat. Acad. Sci. Wash. **15**: 82–85

MOSES, MILDRED S., and METZ., CHARLES W., 1928 Evidence that the female is responsible for the sex ratio in Sciara (Diptera). Proc. Nat. Acad. Sci. Wash., **14**: 928–930.

SCHRADER, FRANZ, 1928 Die Geschlectschromosomen. Zellen-u. Befrucht. I. 194 pp. 43 figs. Berlin: Gebrüder Borntraeger.

SCHULL, A. F., 1929 Determination of types of individuals in aphids, rotifers and Cladocera. Biol. Rev. and Biol. Proc. Cambridge Phil. Soc., **4**: 218–248.

WILSON, E. B., 1925 The cell. 1230 pp. New York: Macmillan Co.

WITCHI, E., 1929 Bestimmung und Vererbung. des Geschlects bei Tieren. Handb. d. Vererbungsw. II. 115 pp. 95 figs. Berlin: Gebrüder Borntraeger.

STEP ALLELOMORPHISM IN *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION

In the fall of 1927, after publication of MULLER'S well known results, the Genetics Laboratory of the MOSCOW ZOOTECHNICAL INSTITUTE, under the guidance of Professor SEREBROVSKY, undertook a series of experiments on the action of X-rays on mutation and on some other special problems of interest to our laboratory.[2] The results of these experiments were published in the *Journal of Experimental Biology*[3] and reported by Professor SEREBROVSKY at the meeting on Genetics and Selection in Leningrad; therefore they will not be discussed at length in this paper. Only one phenomenon of great importance which was described in the former paper will be examined in connection with the present work.

Out of a great number of mutations, which took place in *Drosophila melanogaster* subjected to X-rays, DUBININ discovered two genes of the type of "scute" (reduction of bristles on different regions of the body). A careful examination disclosed that they were neither identical with the previously known mutation scute (according to our terminology scute[1]) nor with each other. Although similar in some characters, they possess well determined differences. In other words, the mutations discovered by DUBININ are not repeated mutations in the strict sense of the word. The distinctive characters of each are as follows:[4]

Scute[1] (PAYNE 1920) reduces the bristles of the head, the sternal, abdominal and scutellar bristles and the first notopleural bristle on each side.

Scute[2] reduces the sternal and scutellar bristles and in some measure the humeral bristles of both sides.

Scute[3] reduces the bristles of the head, abdomen, sides, the frontal pair

[1] Reported at the All-Union Meeting of Genetics and Selection, 1929.

[2] This work was subsidized by the ALL-UNION COMMUNISTIC ACADEMY. It gives the author a special pleasure to express his profound appreciation to this institution for the unremitting and generous assistance shown to our work.

[3] A. S. SEREBROVSKY, N. P. DUBININ, I. J. AGOL, V. H. SLEPKOV and V. E. ALTSHULER, "Mutations by X-rays in *Drosophila melanogaster*," Journal of Experimental Biology, series A. V. 1V, 1928. (U.S.S.R.)

[4] Mutations s_c^1, s_c^2, s_c^3 are numbered according to the chronological order of their discovery.

of dorsocentral bristles and the two bristles between the first and second pair of legs. Besides, scute[3] is characterised by a diminished viability and allows the production of practically no homozygous females.

In compounds, the different types of the gene scute result in a partial reversion to the wild-type, revealing only those characters which are common to both genes present in the compound, while characters specific of each mutation disappear. This fact proves beyond doubt that the two genes are not quite allelomorphic.

The varied combination of characters in different mutations of the scute type made possible a differentiation of the region of the chromosome, occupied by the different transgenes of the scute type, into separate districts, as well as a planning of this region. Theoretical considerations and experimental data led H. P. DUBININ to the establishment of the following order of step-wise allelomorphs of the type scute:

yellow,
scute,[3]
scute,[1]
scute.[2]

The data discovered by DUBININ bear on one of the most important problems in genetics,—the structure and nature of the gene. A further investigation in this direction is most important, especially because the material used by DUBININ (only three step-wise allelomorphs of the type scute) was not sufficient to solve such a fundamental problem, however important DUBININ's general scheme may seem.

A comparative examination of a maximum number of transgenes of the type scute is necessary for a final solution of this problem. MULLER's method promised a sure success in this respect, although MULLER himself, in connection with his work on reverse mutations, mentions his failure as well as that of other investigators to obtain a transgene of type scute from its normal allelomorph by means of X-rays. This fact seems to be accidental, which is well shown by the results obtained by DUBININ and myself, and quite recently by GAISSINOVICH of our laboratory, who discovered scute[5] (reduction of the sternal and scutellar bristles) and by SEREBROVSKY, who discovered scute[6] (reduction of ocellar, postvertical, first and second orbital and first notopleural bristles). This mutation was chosen by our laboratory for investigation, because of the possibility of very accurate determination of its manifestation. The difference in reduction of bristles in different allelomorphs is easily measured and facilitates the work. In this respect the mutation of type scute is to be preferred to many other mutations.

Males, one to two weeks old with the "signal gene" yellow in the sex chromosome, were chosen as objects for roentgenization. The therapeutic tube of COOLIDGE with a tungsten anticathode and a 1 mm aluminum filter was used for the purpose. A 50 K.V. and 5 m. amp. current was used, the flies were placed at a distance of 16–17 cm from the anticathode and subjected to roentgenization. After the exposure to X-rays, they were crossed with virgin females of 2 types: double-X mainly *yy* and partly $s_c w$–$s_c w$, and in a few cases with females with one X chromosome of $s_c\ t\ v\ s_w\ B\ C_1$ structure.

TRANSGENE SCUTE.[4] THE CHARACTERISTICS AND LOCATION OF THE GENE

In one of the cultures, obtained from crossing a yellow male exposed for two hours to X-rays, and a *yy* female, a yellow male with a considerable reduction of bristles of type scute was discovered.

Investigation proved that this mutation was not a repeated mutation in the strict sense of the word. Even though it possessed characters similar to those of the previously known mutations scute,[1] scute[2] and scute,[3] it differed from them in some respects. The new mutation, named scute,[4] is manifested phenotypically in the following way (figure 1). Gene scute[4] reduces the bristles on the head with the exception of the first verticals, reduces the scutellar bristles, the bristles of the sides with the exception of

TABLE 1

Grade of reduction of bristles on different parts of the body of the scute[4] males.

NAME OF BRISTLES	PERCENT OF PRESENCE	PERCENT OF ABSENCE
Ocellars	0	100
Postverticals	11, 8	88, 2
Orbitals	0	100
Vertical I	100	0
Vertical II	0	100
Dorsocentrals	100	0
Scutellars	0	100
Humerals	0	100
Presuturals	0	100
Notopleural I	8, 82	91, 18
Notopleural II	96	4
Supraalars*	100	0
Postalars*	61, 76	38, 24
Bristles between the first and the second pair of legs	0	100

* In the counts of the supra- and postalars there were taken in view only the anterior (large) bristles, and not the posterior (small) ones.

the second notopleural, one supraalar bristle on each side and also acts on the bristles between the first and second pair of legs. The bristles on the abdomen and on the sternites in particular, are also greatly reduced. All these characters are constant and their combination is characteristic of scute[4] only. The bristles on various parts of the body of a large number of flies of different generations were counted. Digressions from the above mentioned characteristics in the mutation discovered were so negligible as

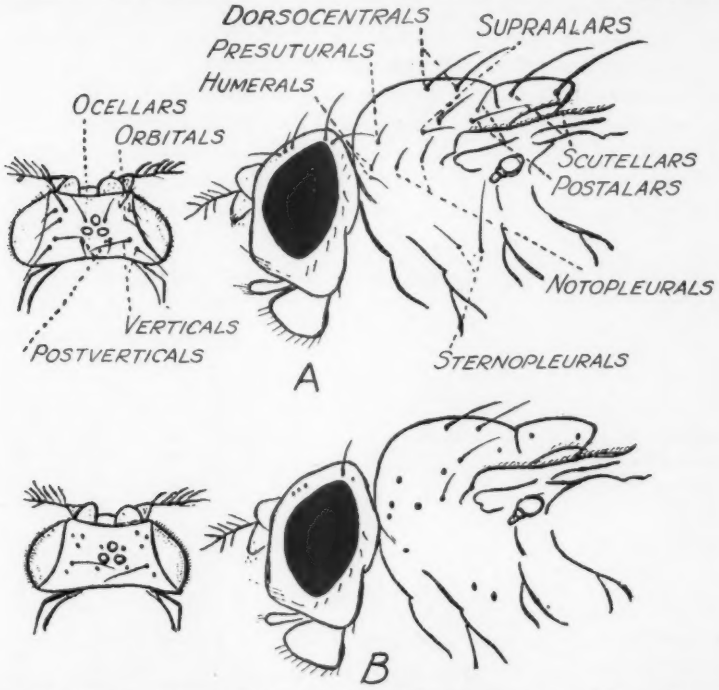

FIGURE 1.—Top view of head and side view of *Drosophila melanogaster*. A, wild-type; B, mutation scute.[4] The positions of absent bristles are indicated by dots, but no such marks are actually present on the flies.

to be of no account. The table on page 256 shows the degree of reduction of bristles on different parts of the body in the male scute[4] (table 1).

Besides, the number of bristles on each of the three hind sternites on the abdomen of the male can be expressed on the average by 6.4 in the last one, then 6.21 and 6.18, which constitutes 33 percent of presence and 67 percent of absence in comparison with the average number of bristles on

the corresponding sternites of the abdomen of a normal male. The number sternopleurals on both sides approaches the normal, if we do not take into consideration the considerable reduction in size and the frequent absence of 2 large bristles. It is not possible to determine accurately the percentage of presence and absence of the large sternopleurals, since on account of their diminution in size, they cannot be distinguished from the other sternopleural bristles.

The comparative study of separate allelomorphs of type scute reveals the complexity of each: separate characters both recur and disappear in various combinations of different scute allelomorphs. Scute^4 can be said to summarize the actions of all of its other allelomorphs (sc^1, sc^2, sc^3) with the exception of the action on the frontal dorsocentral bristle (scute^3) and on the wings (scute^2).

If scute^4 were a partial allelomorph in relation to the previously known transgenes of type scute, then only the coinciding, homozygous districts

TABLE 2

Grade of reduction of bristles in the males and females of different mutations of the type scute.

BRISTLES	ABSENT BRISTLES IN PERCENTS							
	$\sigma^{3}sc^{1}$	$\varphi\frac{sc^{1}}{sc^{1}}$	$\sigma^{3}sc^{2}$	$\varphi\frac{sc^{2}}{sc^{2}}$	$\sigma^{3}sc^{3}$	$\varphi\frac{sc^{3}}{sc^{3}}$	$\sigma^{3}sc^{4}$	$\varphi\frac{sc^{4}}{sc^{4}}$
Ocellars	98	95	0	0	44	Lethal	100	100
Postverticals	91	61	12, 5	6	97	Lethal	88, 2	81
Orbitals I and II	99	82	2	0	83, 5	Lethal	100	100
Orbitals III	0	0	1	0	48, 0	Lethal	100	100
Vertical I	0	0	1	0	82	Lethal	0	0
Vertical II	0	0	0	0	41	Lethal	100	100
Dorsocentrals	0	0	0	0	76	Lethal	0	0
Anterior scutellars	71	30	70, 5	37	8	Lethal	100	76
Posterior scutellars	91	90	91, 5	85	2	Lethal	100	77
Humeral I	0	0	13	12	5	Lethal	100	100
Humeral II	1	0	38	37	35	Lethal	100	100
Presutural	0	0	0	2	70	Lethal	100	83
Notopleural I	98	93	0	0	94	Lethal	91	43
Notopleural II	0	0	0	0	17	Lethal	4	0
Supraalars	0	0	0	0	53	Lethal	0	0
Postalars	0	0	0	0	25	Lethal	38, 24	30
Bristles between the first and the second pair of legs	0	0	0	0	100	Lethal	100	100

would be typically revealed in their compound with it, while the non-coinciding heterozygous districts should bring out the wild-type. In other words, only those characters common to both members of the compound

must be revealed, while differing mutational characters must disappear. The experiments support this theory entirely. The next two tables illustrate the results obtained by me (tables 2 and 3). Table 2 shows the degree of reduction of bristles in various parts of the body of different mutants of type scute. Table 3 shows the degree of similar reductions in different compounds of the same types.

TABLE 3

Grade of reduction of bristles in the compounds of scute^4 with other genes of the type scute.

BRISTLES	ABSENT BRISTLES IN PERCENTS				NOTE
	$\frac{sc^4}{sc^1}$	$\frac{sc^4}{sc^2}$	$\frac{sc^4}{sc^3}$	$\frac{sc^4}{sc^4}$	
Ocellars	96	0	100	100	In the counts of the supra- and postalars only the posterior supraalers, and the anterior postalars were considered.
Postverticals	89	6	92	81	
Orbitals I and II	100	3, 5	100	100	
Orbitals III	0	0, 5	86	100	
Vertical I	0	0	0	0	
Vertical II	0	0	88	100	
Dorsocentrals	0	0	0	0	
Anterior scutellars	68	80	7	76	
Posterior scutellars	85	93	5	77	
Humeral I	8	4	70	100	
Humeral II	0	0	97	100	
Presutural	1	0	90	83	
Notopleural I	98	0	81	43	
Notopleural II	0	0	9	0	
Supraalars	4	0	2	0	
Postalars	24	0	45	30	
Bristles between the first and the second pair of legs	18	29	100	100	

The tables given above show that on the area, where the allelomorphs of the compound have different phenotypic manifestations, reversal toward the normal always takes place. Scute^1 in compound with scute^4 counteracts the reduction of the last orbital bristles, second vertical, both humeral, presutural, second supraalar, and diminishes considerably the percentage of reduction of postalars and of bristles between the first and second pair of legs (100 percent–18 percent). Scute^2 in compound scute^4/scute^2 counteracts the reduction of all bristles on the head, all side bristles with the exception of the humerals, and likewise lowers considerably the percentage of reduction of bristles between the first and second pair of legs (from 100 percent to 29 percent).

Scute^3 in compound scute^4/scute^3 diminishes in a marked degree the percentage of reduction of scutellars (from 100 percent to 6 percent), while losing under the influence of scute^4 its ability to reduce the dorsocentral bristles.[5]

Besides, the reduction of bristles on the sternites in all mutations type scute should be noted. The total number of bristles on the first three sternites is expressed as follows: in the male scute^1—26.4, in the female—scute^1/scute^1—32.01; in the male scute^2—15.6, in the female scute^2/scute—16.6; in the male scute^3—31; in the male scute^4—28.75; in the female scute^4/scute^4 —30.44; in compound scute^4/scute^1—28.94; scute^4/scute^2—22.1; scute^4/scute^3—29.72. The entire quantity of sternopleural bristles on both sides is: in the male scute^1—16.86, in the female scute^1/scute^1 —17.5; in the male scute^2—16.4, in the female scute^2/scute^2—17.1; in male scute^3 —9.8, in the male scute^4—15.5; in the female scute^4/scute^4 —15.8.

In this way, the comparative study of the action of each separate transgene—s_c^1, s_c^2, s_c^3, and s_c^4 (table 2) together with the action of each in compound with transgene s_c^4 (table 3), leaves no doubt that the transgenes examined are not altogether allelomorphic in relation to each other. The reversal to the wild (normal) type always follows in that district, where the separate members of the compound exhibit different phenotypical manifestations (figure 2).

The comparative study of compounds of scute^4 and the other transgenes of type scute permits the establishment of a row of basic (elementary) districts, which enter as components in some transgenes and are not acted upon in others. As an example, the fact may serve that the area which conditions the reduction of ocellar, orbital and the first notopleural bristles is established in the following manner. Compound scute^4/scute^6 exhibits the manifestation of ocellar, postvertical and the first and second orbital and first notopleural bristles; compound s_c^4/s_c^2 exhibits a reversal to normal of ocellar, first and second orbital and first notopleural bristles, but prevents the manifestation of post-vertical bristles. It follows that the district acting on the postvertical bristles can be combined independently of the districts acting on the ocellar, orbital and notopleural districts. A comparative analysis of all compounds of type scute does not allow us yet to divide this district into more elementary ones, since in all of these compounds, the reduction of bristles, conditioned by this district, manifests itself always as a whole. By means of a comparative analysis of compounds of scute^4 and the other transgenes of type scute (in this case we

[5] The description of homozygotic females s_c^4, will follow. In the table the percent of reduction in such females is given for comparison with other compounds.

call them analysers) one can establish the following seven elementary districts in transgene scute[4]:

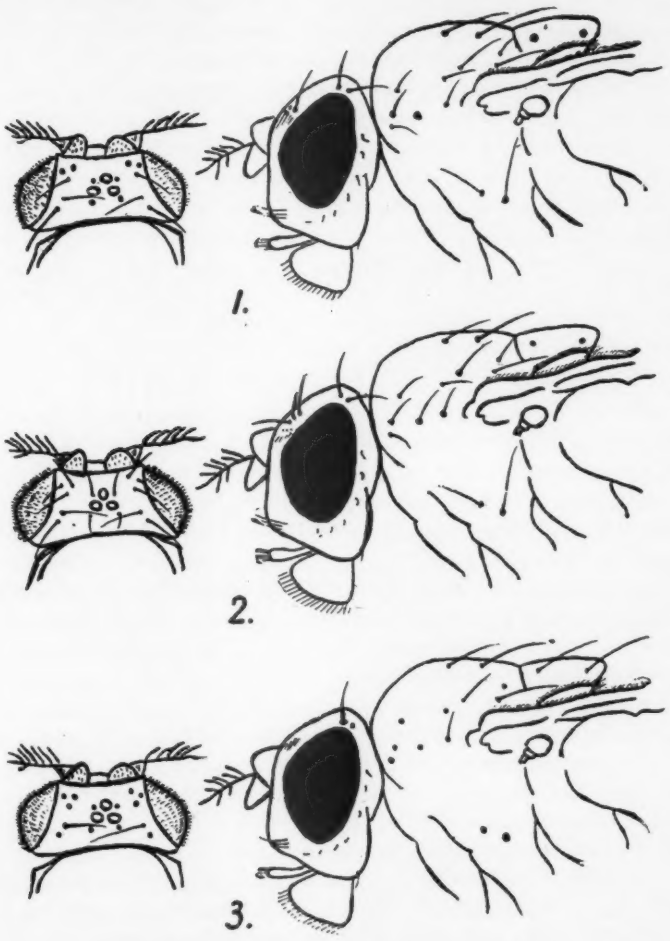

FIGURE 2.—Compounds sc^4/sc^1 (1), sc^4/sc^3 (2), sc^4/sc^3 (3).

first district, determining the reduction of ocellar, first and second orbital and first notopleural bristles (analysers sc^4/sc^6 and sc^4/sc^2); second district, determining the reduction of postvertical bristles (analysers sc^4/sc^2 and

s_c^4/s_c^6); third district, determining the reduction of the second vertical and supraalar bristles (analysers s_c^4/s_c^3, s_c^4/s_c^1, s_c^4/s_c^2); fourth district, determining the reduction of scutellar bristles (analysers s_c^4/s_c^5 and s_c^4/s_c^3); fifth district, determining the reduction of humeral bristles (analysers s_c^4/s_c^2, s_c^4/s_c^6 and s_c^4/s_c^3); sixth district, determining the reduction of sternal bristles (s_c^4/s_c^3 and s_c^4/s_c^5); seventh district, determining the reduction of bristles between the first and second pair of legs (s_c^4/s_c^3).

The study of females homozygous for scute[4] revealed the fact that the percentage of reduction of bristles in such females in comparison with their degree of reduction in males scute[4] is lower (table 2). Such a decrease in the quantity of bristles can be observed in all females homozygous for every gene of type scute. This phenomenon has not yet been adequately explained. We are not able to say at present whether this phenomenon is determined by the sexual dimorphism of Drosophila or whether it depends on other causes. A similar decrease in the reduction of bristles in the homozygous condition can be observed in the mutation recently discovered by N. P. DUBININ.[6]

The determination of the position of scute[4] in the series of step allelomorphs of type scute in the usual way, that is by calculating the percentage of crossing over, seems a difficult problem to solve, inasmuch as we are not able to obtain a crossing over between the separate transgenes of type scute.[7]

I did not have at my disposal triploid females producing a certain percentage of crossing over, between yellow and scute. This problem, however, can be solved in another way. I attempted to determine the position of scute[4] by means of a comparative analysis of its characters and the characters of other transgenes scute. The phenotype of transgene scute[4] is a resultant phenotype of the separate districts of all of its step allelomorphs, except the district which influences the dorsocentral bristles. We now come to the conclusion that the region occupied by scute[4] is a sum of the other regions, occupied by all other known genes of type scute. But scute[4] does not include all of the transgene scute[2], which is manifested phenotypically in wrinkled, spoon-like wings, in addition to reduction of bristles; that is, the object of its action in this case is more extensive than that of scute[4]. This fact allows us to assume that the right end of gene scute[2] may have a common district with transgene spoon, lying at locus

[6] This mutation (named s_{Md}) is manifested phenotypically as follows: coarse and diminished eyes, reduction and doubling of bristles. This transgene is situated in the sex chromosome in the region of Notch or somewhat to the left of it.

[7] Investigation of crossing over showed that a roentgenised chromosome, bearing scute,[4] modifies it noticeably. This phenomenon is now being studied.

0.2 on the map, and possessing a similar manifestation on the wings. We do not observe such an action on wings in scute^4, consequently it does not extend so much to the right. The right end of gene scute^4 lies to the left of the right end of scute,[2] hence does not include all gene scute^2. In such a manner, we arrive at the following determination of the position of transgene scute^4. It extends over the greater part of transgenes, scute^1, scute^3, and scute^2, its right end approaching transgene spoon, without touching it. The position of scute^4 in the series of step allelomorphs, may be expressed in the following scheme (figure 3).

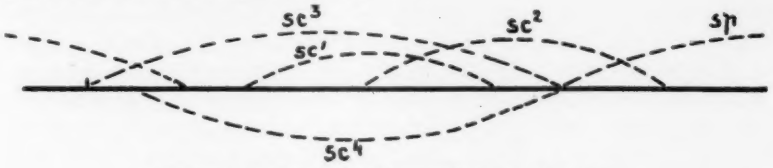

FIGURE 3.—Series of step allelomorphs s_c^3, s_c^4, s_c^1, s_c^2, s_p.

The insufficient data regarding additional cases of step allelomorphs, does not yet allow us to build up an accurate scheme, which would include all known districts. As we obtain additional cases of partial allelomorphs type scute, we will be able to determine definitely the boundaries of separate districts, to study accurately the special position of these districts and to build up a plan of the basegene. Consequently, our efforts should be directed towards getting new mutations type scute. One more mutation in the sex chromosome of Drosophila has been discovered by N. P. DUBININ, related phenotypically to mutations type scute (reduction of bristles of different parts of the body). If this transgene should really prove to be a new partial allelomorph type scute, we will have in addition to yellow and spoon, seven allelomorphs type scute.[8] Such a series of partial allelomorphs will help us in the solution of the problem of the structure of genes.

SOME THEORETICAL CONCLUSIONS

The results concerning the phenomenon of step allelomorphism described above lead us to very important conclusions in regard to the nature and structure of the gene. We first arrive at the conclusion that the gene is not, at least not always, something homogeneous, indivisible, but may be represented by a complex organization, composed of separate districts joined with each other, each possessing its specific action. The connection be-

[8] The mutation discovered by N. P. DUBININ has since been found to be a new step allelomorph type scute. It is recorded in our laboratory as scute.[7] As this goes to press, the count of mutations of the scute locus totals approximately three dozen.

tween districts is constant in each separate transgene. In other words each given transgene possesses a definite number of districts in some way joined with each other. Such are, for instance, the different combinations of districts in various allelomorphs of type scute, which arose as mutations in individuals of the wild-type.

The theory of the linear arrangement of genes is based on the phenomenon of linkage which was investigated by means of crossing over. This method unfortunately could not be used, since there is no practical way of obtaining crossing over between the separate districts, because of their very close proximity to each other. Our investigation of the special position of step allelomorphs was based on the facts of partial allelomorphism and on the consideration of the basegene, as a complex structure made up of separate components closely joined with each other. The character of the attachment between the separate districts has an important bearing on the problem of the special structure of the gene. The action of each district doubtless depends on the action of the chromosome as a whole, but at the same time it is independent to such a degree that it retains its individuality in every genotypic combination. The various combinations of separate districts in different scute-allelomorphs and the constant manifestation of each district, regardless of the other components in the combination, leaves no doubt that each separate district which goes into the composition of every transgene, possesses its comparatively independent action, manifested in the formation of specific characters. All the cases of partial allelomorphism, of type scute, described above and the behaviour of every pair of these allelomorphs in compound demonstrate this clearly. An example of this is the manifestation in compounds of homozygous districts only, and the repression of heterozygous, regardless of the other parts of the allelomorph in which they are included. The separate districts of the transgenes described behave in such a way, that knowing them, it is possible to calculate beforehand and predict the other possible mutations of a given type. The possibility of mutation scute^4 was among those which, based on the study of separate districts of previously known transgenes scute^1, scute^2, scute^3, was predicted by SEREBROVSKY. Just as we are obliged to attribute to every gene a relative independence in its manifestations and a strictly definite localization, regardless of the fact that its action is doubtless connected with the action of the entire chromosome apparatus, in the same way, we must also attribute to separate districts of genes a relative independence of action and a definite localization.

The conception of the gene, as of a complex structure, composed of separate comparatively independent districts, makes it necessary to revise

the traditional view of allelomorphism. We may consider as allelomorphs not only those genes which coincide throughout their extent and do not allow any reversal to the wild-type in compounds, but also those genes which coincide only in one or several districts and which produce a partial reversal to the normal (wild) type in compounds. The presence of a large number of comparatively independent districts in the basegene leads us to conclude that the possibility of a strict repetition of changes in all districts is very small; it follows that the newly arising allelomorphs must in most cases be partial or incomplete, some of their districts coinciding with those of other transgenes. Such are, for example, the allelomorphs scute^1, scute^2, scute^3, scute^4, scute^5, scute^6 and scute^7. The three allelomorphs type dachsous belong to this category. For instance, the transgene dachsous^1 includes the districts of the chromosome acting both on the crossveins and the shape of wings. The wings remain normal in the mutation dachsous^3, but it includes the district acting on the ovary which was left normal by the former transgene. The compound between these two transgenes manifests only those characters which are related to the district of the chromosome included by both transgenes. All other allelomorphs should be thoroughly investigated in this direction, as well as the so-called repeated mutations. We can assume theoretically a long stair of allelomorphs, which gradually pass from one gene into another and extend throughout the entire length of the chromosome. Two different genes not allelomorphic in relation to each other may be partial allelomorphs in relation to another gene, for instance, spoon and scute^1, in relation to scute^2.

I will indicate another important conclusion drawn from the phenomenon of step allelomorphism. It has to do with crossing over. If the entire chromosome is continuous, that is, if one gene passes directly into another, then the crossing over may pass only through the gene and not between genes, since step allelomorphism exludes any spaces between genes.

The phenomena described in this paper have a bearing on the question of multiple allelomorphs. Considering new data, the old view, that all allelomorphs of one type, obtained as a result of mutation, correspond only to one normal allelomorph, seems purely formal. In reality, as follows from the phenomenon of partial allelomorphism described above, each definite transgene corresponds to its specific normal gene. In other words, not one normal gene corresponds to all allelomorphs of a given type, but as many normal genes as there are allelomorphs. Normal allelomorphs differ from each other in the same degree as do their corresponding transgenes. The

normal allelomorph of scute[4] is not the same as that of scute[3], scute[2], or scute[1]. The latter all represent different regions in the normal chromosome.[9]

LITERATURE CITED

ALTENBURG, E., 1928 The limits of radiation frequency effective in producing mutations. Amer. Nat. **62**: 540–545.

DUBININ, N. P., 1929 Allelomorphentreppeen bei *Drosophila melanogaster*. Biol. Zbl. **49**: 328–339.

GAGER, C. S., and BLAKESLEE, A. F., 1927 Chromosome and gene mutations in Datura following exposure to radium rays. Proc. Nat. Acad. Sci. Wash. **13**: 75–79.

GOODSPEED, T. H., and OLSON, A. R., 1928a The production of variation in Nicotiana species by X-ray treatment of sex cells. Proc. Nat. Acad. Sci. Wash. **14**: 66–69.

1928b Progenies from X-rayed sex-cells of tobacco. Science **67**: 46.

HANCE, R. T., 1928 The reaction of homozygous and heterozygous adult characters under the influence of X-rays (Abstr) Anat. Rec. **37**: 176.

HANSON, F. B., 1928a The effects of X-rays on productivity and the sex ratio in *Drosophila melanogaster*. Amer. Nat. **62**: 352–362.

1928b The effects of X-rays in producing return gene mutations. Science **67**: 562–563.

HANSON, F. B., and HEYS, F. M., 1928 The effects of radium in producing lethal mutations in Drosophila. Science **68**: 115–116.

MULLER, H. J., 1927a Artificial transmutation of the gene. Science **66**: 84–87.

1927b The problem of genic modification. Proceedings of the Fifth International Congress, Supplementband of the Z. indukt. Abstamm.-u. VererbLehre **1**: 234–260.

1928 The measurement of gene mutation rate in Drosophila, its high variability and its dependence upon temperature. Genetics **13**: 279–357.

PATTERSON, J. T., 1928 The effect of X-rays in producing mutation in the somatic cells of *Drosophila melanogaster*. Science **68**: 41–43.

SEREBROVSKY, A. S., 1926 The influence of the gene "purple" on crossing over between the genes "black" and "cinnabar" in *Drosophila melanogaster*. J. Exp. Biol. (U.S.S.R.) T **2**: 55–100.

SEREBROVSKY, A. S., DUBININ, N. P., AGOL, I. I., SLEPKOV, V. N., and ALTSHULER, V. E., 1928 The production of mutations by X-rays. J. Exp. Biol. (U.S.S.R.) **3–4**: 161–180.

STADLER, L. J., 1928a Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. Wash. **14**: 69–75.

1928b Mutation in barley induced by X-rays and radium. Science **68**: 168.

TIMOFÉÉF-RESSOVSKY, 1929 Rückgenovariationen und die Genovariabilität in verschiedenen Richtungen. Arch. EntwMech. Org. **115**: 620–635.

WEINSTEIN, A., 1918 Coincidence of crossing over in *Drosophila melanogaster*. Genetics **3**: 135–173.

1928 The production of mutation and rearrangements of genes by X-rays. Science **67**: 376–377.

WHITING, P. W., 1928 The production of mutation by X-rays in Hadrobracon. Science **68**: 58.

[9] This possibility was predicted by A. S. SEREBROVSKY in 1926 in his paper on "The effect of gene purple on crossing over between genes black and cinnabar in *Drosophila melanogaster*."

THE SIDE-CHAIN THEORY OF THE STRUCTURE OF THE GENE[1]

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INTRODUCTION

In 1923, after three years as research assistant to Professor CHARLES ZELENY during the time that the rates of mutation of the bar and ultrabar genes were being determined, it occurred to the writer that mutation of these genes may be explained as the gain or loss of certain discrete particles or units within the gene. This idea was developed and presented as a thesis in 1924 and a brief abstract published (THOMPSON 1925). During the past six years it has been possible to extend the theory by using data published by numerous geneticists.

[1] Contribution No. 396 from the Zoological Laboratories of the UNIVERSITY OF ILLINOIS, and the ILLINOIS STATE NATURAL HISTORY SURVEY.

This conception pictures the gene as consisting of a main particle firmly anchored in the chromosome with varying numbers of one or more kinds of other particles attached. The main particle is called the protosome and the attached particles the episomes. (In the article just referred to, these particles were called the "base" and the "modifier," terms which unfortunately are confusing since "modifier" is already used in genetics to mean accessory factor and "base" has also a wide range of technical meanings in the different branches of science.) Gene mutation is due most frequently to the loss of one or more episomes from the protosome and less frequently to the addition of episomes. When two or more episomes of the same kind are present in a gene, they are attached one to the other so as to form a side-chain. Different kinds of episomes do not enter into the same side-chain, but each kind has its own attachment with the protosome. Varying numbers of the same kind of episome produce "quantitative" series of multiple allelomorphs. Epistatic series and allelomorphs which are "qualitatively" different involve variations in the numbers of two or more kinds of episomes. Shifting of the position of an episome or chain of episomes on the protosome may account for changes in the rate of mutation or for different linkage relations. Episomes may be transferred occasionally, not only from one protosome to its homologue in the other chromosome, but also from one locus to another. The writer visualizes the genetic chromosome as the conventional string of beads used to demonstrate crossing over, except that almost every bead bristles with side-chains of episomes.

DIRECTION AND FREQUENCY OF MUTATIONS

There is a wide variety of types of eye in *Drosophila melanogaster* depending on genetic conditions at the bar locus in the X-chromosome. The eyes differ primarily in the number of facets, or ommatidia, which may be counted and accurate quantitative comparisons made. The individual ommatidium remains almost constant in size in the different types of eye under ordinarily good culture conditions, so that facet counts give an expression of area.

Origins and mutations of the genes of the bar series

All bar-eyed flies and all of the bar allelomorphs are directly descended from a single bar male found by TICE in 1913 in an experiment involving only normal wild-type eyes. Among the millions of Drosophila with wild-type eyes that have been examined since 1913, no recurrence of the bar mutation has been reported.

In 1917, MAY reported eleven occurrences of the reverse mutation, that

is, from bar to full, or round eye. His tests as well as those of a number of subsequent workers have failed to show that the full eye derived from bar by reverse mutation is in any way different from the wild-type eye.

In 1920, ZELENY described a new allelomorph, called ultrabar (double-bar of STURTEVANT), which was found in a homozygous bar stock. Whereas homozygous bar eyes ordinarily have about nine percent as many ommatidia as the wild-type eye, this new allelomorph has only about three percent as many facets. Females heterozygous for bar and full have an intermediate eye with about half as many facets as are found in the wild-type eye. On the other hand, heterozygotes of ultrabar and full, while still inter-

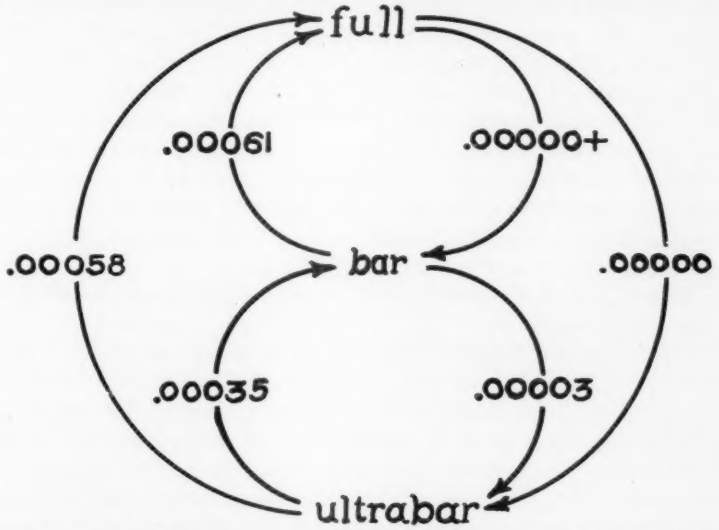

FIGURE 1.—Rates of mutation in the bar series of multiple allelomorphs. (After ZELENY 1921).

mediate, have only about six percent as many facets as full. Heterozygotes of ultrabar and bar are likewise intermediate, with about four percent as many facets as full. ZELENY'S tests indicated that ultrabar fulfills the requirements of allelomorphism with bar and full. There are no apparent "qualitative" differences between full, bar, and ultrabar which would prevent their consideration as a "quantitative" series of multiple allelomorphs in the order named. The ultrabar allelomorph not only mutates back to bar, from which it was derived, but also mutates directly to full without passing through the bar condition. The rates of mutation between these allelomorphs as determined by ZELENY (1921) are given in figure 1.

STURTEVANT (1925, 1928) explains the origin of ultrabar as due to unequal crossing over at the bar locus resulting in two bar genes lying next each other in the linear series of the genes. According to his explanation, reverted bar and presumably wild-type full, also, are not represented by an allelomorph at the bar locus; furthermore, homozygous bar should produce full and ultrabar mutants in approximately equal numbers. This has not been realized. ZELENY's (1921) data show that among 85,008 bars examined the fulls outnumber the ultrabars 17:1. (During the course of this experiment, numbers of flies with low facet counts were tested and found not to be ultrabar.) As STURTEVANT has pointed out, by the inclusion of certain untested flies with unusually low facet numbers this ratio is lowered to 6.5:1. More recently (1928) he reports 8 full mutants and 2 ultrabar mutants from homozygous bar; a ratio of 4:1. While the true rate of mutation from bar to ultrabar may be considerably in excess of the 0.00003 given by ZELENY, it is difficult for me to believe that I overlooked numbers of ultrabars approximating the fifty or so full mutants which were found.

The difficulties of recognition of bar-ultrabar and full-ultrabar heterozygotes among an ultrabar population, as well as the preponderance of male mutants, make it seem likely that female mutants from ultrabar to bar were all overlooked and that part of those from ultrabar to full went unrecognized, so that the rates given in figure 1 (0.00035 and 0.00058) are probably too low.

STURTEVANT and MORGAN (1923) and STURTEVANT (1925, 1928), by a most ingenious and critical series of experiments, have shown that mutation at the bar locus is associated with crossing over in that region. A most important result of these experiments is that STURTEVANT by genetic manipulation apparently has conclusively demonstrated that the germinal difference at the bar locus resulting in a full eye on the one hand and a bar eye on the other is exactly equal to the germinal difference between a bar eye and an ultrabar (double-bar) eye.

Evidence from other sources shows that mutations at the bar locus may occur in the female at other stages in the life history than at the maturation divisions, and in the male as well as in the female. HANSON (1928a, 1928b) reported 9 reversions of bar to full among the offspring of irradiated bar males. It seems improbable that these mutations were associated with crossing over, since crossing over does not occur in the male in Drosophila. Other evidence of HANSON's (1929) shows that irradiation has its effect on mature spermatozoa, which are haploid, thus making any possible association with crossing over quite out of the question.

PROFESSOR ZELENY has kindly permitted me to present here unpub-

lished data of his concerning an instance of coincidence of mutation from bar to full and from bar to ultrabar which occurred in 1922. A virgin female of an inbred white bar stock was mated to a brother and gave 105 offspring in 4 bottles. In bottle 3 of this mating there were 8 females and 12 males. Of these 8 females one appeared to be a heterozygote between bar and full, and another was as low in appearance as ultrabar, while the rest were typical bars. This full-bar heterozygote was not tested, but numerous others have always proved to be what they seemed, that is, heterozygotes for bar and full. The low female, when mated to a bar brother, gave only two offspring and a third pupa which did not hatch. One of the two offspring was a bar male with 83 facets and the other a male with 30 and 32 facets in the two eyes, respectively, at room temperature.

A similar coincidence of mutation happened in the spring of 1920 in the course of work with the character erect wing. A white bar erect female, which was homozygous for the white and bar factors and heterozygous for the sex-linked erect factor and for an autosomal factor which makes erect dominant (THOMPSON 1921), was mated to a wild-type male. Neglecting erect and its accessory factor, the expected classes were full-bar heterozygotes with red eyes and white bar males. The offspring consisted of 49 females and 47 males, respectively, for the expected classes and *one red full-eyed female* and *one red-eyed female classified as low bar*. Up to the time of the above-mentioned coincidence of mutation, this case could not be explained, but it now seems explicable in similar terms; that is, a bar female produced two mutant eggs, one of which bore a full and the other an ultrabar gene.

That the process which causes bar to mutate to full is associated with the process that produces ultrabar seems certain; for if they occurred independently, the probability of obtaining the two in a bottle of twenty flies would be one in several millions. While it has been shown by STURTEVANT (1925, 1928) that these two processes ordinarily are accompanied by forked-fused crossing over, these cases of coincidental mutation could not have involved crossing over; for in order to result in two mutants, the change in each case must have taken place one or more cell generations before the maturation divisions.

Another interesting case of coincidence of mutation came to light in November, 1923. An ultrabar forked female of a pure stock was mated to an ultrabar forked brother. The offspring consisted of 9 ultrabar forked females, 19 ultrabar forked males, *and one full forked female*. This full female could not have been the result of contamination because no forked full stock had been in the building for months, and no strays of this kind were in the

fly traps. Neither could she have resulted from the highly improbable fertilization of a mutant egg by a mutant sperm, since mutation in the male is not known to take place except by irradiation. It therefore seems probable that she had received both of her full genes from her mother. This egg, then, must have contained two X-chromosomes and was fertilized by a Y-sperm. In this case the full female should give exceptional offspring due to secondary non-disjunction. She was mated to an ultrabar forked brother and gave 47 forked daughters heterozygous for ultrabar and full, 37 full forked sons, and *one full forked daughter*. She was then mated to a full forked son and, after a few offspring by the previous male, gave all full forked offspring. This correlation of mutation with non-disjunction is all the more significant since non-disjunction is extremely rare in the stocks used. Recent experiments involving over 500 matings did not show a single exceptional offspring that could be due to non-disjunction.

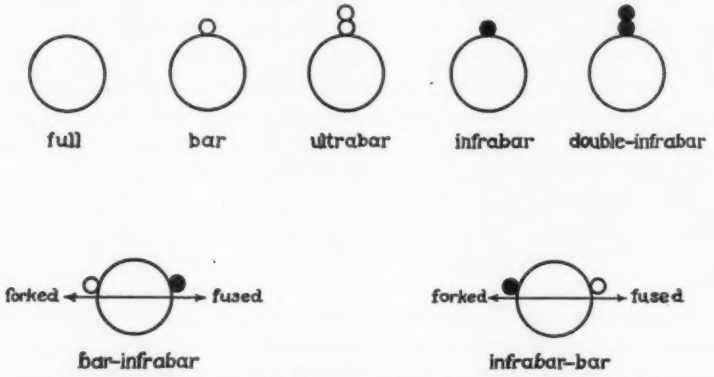

FIGURE 2.—Diagrams of the different genes known to occur at the bar locus.

The mutation phenomena of full, bar, and ultrabar thus far described may be interpreted briefly in terms of the structure of the gene as follows: The original bar mutation arose by the addition of a bar episome to a protosome at the bar locus in a full-eyed fly. This bar episome came from some other locus. The bar episome of the bar gene is frequently broken off, giving reverse mutations to full. When a bar episome is dislodged in a homozygous bar female, it may occasionally attach to the homologous bar gene to produce an ultrabar gene. Those episomes which are dislodged and do not become attached to the homologous gene may either be attached at some other locus or else be resorbed and lost to the germ-plasm. When a bar gene adds another bar episome to produce an ultrabar gene, we infer

that the second episome is attached to the first in a side-chain arrangement because the ultrabar gene loses both its episomes to produce a full mutant as readily as it loses one to produce bar.

STURTEVANT and MORGAN'S demonstration that mutation at the bar locus is associated with forked-fused crossing over is interpreted to mean that crossing over, with its implied intimate contact at the point of breaking, is an effective agency for dislodging episomes or for transferring them to the homologous gene. That crossing over is not the only agency which can dislodge the bar episome is shown by HANSON'S production of mutations from bar to full by irradiating the spermatozoa of bar males. The two instances of coincidence of mutation at the bar locus occurred in the female during the oögonial divisions. Changes in cellular mechanics accompanying non-disjunction seem to have been responsible for the loss of the episomes from the two ultrabar genes of an XX egg.

The genes of the bar series of allelomorphs may be diagrammed as in figure 2.

Origins and mutations of the genes of the infrabar series

STURTEVANT in 1925 reported a new allelomorph of bar which he called infrabar. This mutation appeared as a single male in an experiment where all the fathers were bar and all the mothers had the two X-chromosomes attached, giving 100 percent non-disjunction. This mutation falls in a different category from those being considered in this paper, as it represents a change in the nature of the bar episome, that is, it involves episome mutation and not gene mutation. With this new kind of an episome STURTEVANT has duplicated most of the phenomena described earlier in this paper for the bar series of "quantitative" allelomorphs. Infrabar behaves like bar in that it reverts to full, but at a rate three to four times as great (STURTEVANT 1928). It also produces a new and more extreme allelomorph called double-infrabar, analogous to ultrabar. Just as homozygous bar produces ultrabar less frequently than it reverts to full, homozygous infrabar produces double-infrabar less frequently than it reverts to full. Double-infrabar likewise breaks down to give infrabar or reverts to full directly. Double-infrabar, infrabar, and full constitute a "quantitative" series of allelomorphs paralleling the ultrabar, bar, and full series.

This infrabar series differs qualitatively from the bar series. STURTEVANT (1925) says, ". . . homozygous infrabar is about like bar over round in facet number, but the two types can be separated by a peculiarity common to all the larger infrabar and double-infrabar types, namely, a roughened appearance of the eye, due to irregularities in the rows of facets. This

peculiarity is not present in bar eyes, and is almost completely recessive in bar over infrabar. In infrabar over round (which is not far from round in facet number) the roughness is variable in extent, and may be not at all evident, in which case the type can not be distinguished with certainty from homozygous round. In other stocks, where the modifiers are different, it often happens that infrabar over round is regularly conspicuously roughened and is easily distinguishable from round. This roughness of the eyes may be taken as evidence that the infrabar gene is qualitatively different from bar, rather than being merely a fraction of bar."

KRAFKA (1920) showed that wide differences in facet number result from differences in temperature at which bar or ultrabar larvae develop, approximating 10 percent decrease in facet number for each degree C increase in temperature. ROSELLE KARRER HERSH (1924) showed that full eye is affected in the same direction but to a less extent, being 2½ percent per degree. DRIVER (1926), in a very painstaking analysis of the temperature effect on bar and ultrabar, has shown that with increasing temperatures the rate of facet formation per unit of time during the facet-forming period is not much altered, but that the lower facet numbers result primarily from a shortening of the temperature-effective period.

Further evidence of the qualitative difference between infrabar and bar is found in LUCE's (1926) discovery of the surprising fact that temperature has the opposite effect on infrabar and double-infrabar; that is, increasing temperatures give increasing facet numbers. His analysis shows that with increase in temperature the rate of facet formation during the temperature-effective period, instead of being almost constant as in bar and ultrabar, is increased enormously—so much so that it far more than counterbalances the opposite effect due to a shortening of the temperature-effective period.

The qualitative difference between the bar series and the infrabar series is represented in figure 2 by means of two different kinds of episomes, which, however, have the same structural relations to the protosome.

Composite genes of bar and infrabar

Evidence indicating some details in the relation of episomes and protosomes is found in the manner of origin of certain mutants. STURTEVANT (1925) has shown that when bar and infrabar genes are in the two X-chromosomes of a female a new allelomorph containing both may be produced in a manner analogous to the production of ultrabar or double-infrabar. This compound allelomorph breaks down to give either bar or infrabar mutants, but to date he has reported no mutations from it directly to full. These bar and infrabar mutants occur with a *special relation to*

crossing over. All the bar mutants were associated with crossing over between the forked locus and the bar locus, while all the infrabars were crossovers between the bar locus and fused. In another independently produced composite gene of bar and infrabar the relations were reversed, so that infrabar resulted from crossing over in the first region and bar from the second. He reports thirteen mutants consistent in this relation to crossing over, with no exceptions.

The facts just mentioned are interpreted to mean that the bar and infrabar episomes of these composite genes are not attached in side-chain arrangement but lie *near the left and right poles of the gene*, so that crossing over on the left side breaks off the one (or transfers it to the homologous bar allelomorph), while crossing over on the right side breaks off the other. The difference between bar-infrabar and infrabar-bar genes can be seen in figure 2. While, up to date, STURTEVANT has reported no direct mutations to full from these composite genes, an experiment showing whether or not they produce fulls at a rate approaching their rate of mutation to bar or infrabar would be a test for this postulated structure.

SOMATIC MANIFESTATIONS OF MUTATIONS

In the preceding discussion of the structure of the genes at the bar locus, only the most direct evidence available has been used, that is, evidence showing the origins of the different allelomorphs and the direction and frequency of mutation. Detailed discussion of facet number has been purposely avoided except as it was necessary to recognize the different mutations. All available data, however, do indicate that the gain or loss of one of these germinal units, or episomes, is accompanied by a definite jump in the facet number, and that these different units maintain their identity no matter what combinations they have entered into or what manipulations they have undergone in their past history. It is now proposed to discuss the somatic manifestation of these genes and to point out, in particular, how facet number is determined by the ratio of the number of episomes to the number of protosomes. Before undertaking the exact application of such a formula, however, it is necessary to consider two apparent exceptions, namely, the so-called position effect and sexual dimorphism.

The so-called position effect

STURTEVANT (1925, 1928) has pointed out what he calls the position effect of genes on facet number. According to his unequal crossing over explanation of mutation at the bar locus, it is to be expected that homozygous bar females, with a bar gene in each X-chromosome, would have the

same facet count as full-by-ultrabar heterozygotes, with two bar genes in one X-chromosome and none in the other. When these two are compared he finds that the heterozygote always has fewer facets than the homozygote, the number varying in different stocks from ⅔ to ⅕ as many. The same relation holds when infrabar homozygotes are compared with heterozygotes of full and double-infrabar and when bar-by-infrabar heterozygotes are compared with heterozygotes between full and the composite bar-infrabar allelomorph. Such comparisons, at first, appear to present the same difficulty of explanation on the basis of side-chain structure of the gene as on the basis of unequal crossing over, since the same structural elements are present in both; but there is an essential difference. I am indebted to Professor ZELENY for the suggestion that the difference in facet number shows that the two bar factors (episomes) in an ultrabar gene do not exist as separate entities but are bound up with a third and different member (the protosome) to form a single gene which, with a normal allelomorph (protosome alone) in the homologous chromosome, may be expected to give a different result from that produced by a bar episome on each of two protosomes. If two episomes always gave the same result no matter how they were distributed, there would be no obvious need of postulating the existence of the protosome. Furthermore, if the same facet number always resulted, it would indicate that two allelomorphic genes, although located in two separate chromosomes, could together constitute a genetic unit of a more fundamental nature than a single gene—a situation without parallel among the units of the physico-chemical world. Finally, the absence of "position effect" on bristle number has been shown by STURTEVANT (1928) in a case in which the genes unquestionably exist as separate entities.

Sexual dimorphism

Males of the different allelomorphs at the bar locus have consistently higher facet numbers than the corresponding homozygous females. The sex coefficients in the order of decreasing facet number, and the authority quoted for each, are as follows: full 0.984 (ROSELLE KARRER HERSH 1924); infrabar 0.729 (STURTEVANT 1925); bar 0.791 (KRAFKA 1920); double-infrabar 0.831 (STURTEVANT 1925); bar-infrabar 0.899 (STURTEVANT 1925); ultrabar 0.953 (ZELENY 1920). Since the stocks on which these dimorphism coefficients are based seem to have been more or less closely inbred, these observed differences are brought about by the male having a single dose of the facet-determining factors in the X-chromosome while the female has a double dose of these factors. These differences, however, are

not necessarily due to single and double doses at the bar locus alone; for A. H. HERSH (1929) has demonstrated the existence of plus and minus modifiers of facet number in other parts of the X-chromosome. His findings bear out ZELENY'S (1921) explanation that the marked decrease in sexual dimorphism following inbreeding results from the elimination of differences due to X-chromosome factors affecting facet number.

Sexual dimorphism is greatest in the middle range of facet number, where a more labile condition is also indicated by temperature coefficients and by coefficients of variability. The so-called position effect also falls in this labile range.

The facet numbers determined by genes at the bar locus

Facet number is more closely correlated with the ratio of the number of episomes to the number of protosomes than with the total number of each or with other mathematical relations of these structural elements. If the different facet numbers are determined by quantitative changes in the gene, there should be some mathematical relation between the number of bar episomes and facet number when the number of protosomes is held constant. The males of the bar series, having only three members, cannot be expected to show the relationship as readily as the female series with its six members. The facet counts at 25°C for full eye by ROSELLE KARRER HERSH (1924) and for the other members of the series by A. H. HERSH (1922) have been used because all of them were derived by recent mutation from one stock and should have about the same combination of accessory factors. Since homozygous bar females and full-ultrabar heterozygotes both have two bar episomes, their facet numbers will be averaged, leaving five facet numbers in the female series as follows:

Kind of female		*Number of episomes*	*Facet number*
Ultrabar homozygotes		4	24.5
Bar-by-ultrabar heterozygotes		3	28.1
Bar homozygotes	average	2	49.0
Full-by-ultrabar heterozygotes			
Full-by-bar heterozygotes		1	325.0
Full homozygotes		0	740.0

The interval increases rapidly for the first four members in this facet-number series but is not much larger for the last member than for the one before it. This again involves the consideration of qualitative differences. For instance, if A and B are different kinds of things, A is qualitatively different from the combination of A+B. On the other hand, A+B is quantitatively different from A+2B; and A+2B from A+3B; and so on. Since

the last interval in the above tabulation represents a qualitative difference in the composition of the gene, the full facet count should be excluded from the quantitative series. Analogous results are to be found in a consideration of the physical properties of series of organic chain-compounds, in which the zero, or end-member, of the series does not lie on the curve determined by the other members.

ZELENY (1920b) found an exponential relation to exist in the variations of the facet numbers of the different members of the bar series. KRAFKA, the HERSHES, and DRIVER found the same kind of relation in the facet counts of flies reared at successive intervals of temperature. It is then desirable to express the facet number in this female series in terms of the number of episomes, preferably by a generalized exponential equation, as follows:

$$\log_{10} f(n) = a + bc^n$$

Here the number of episomes is represented by n, and the facet number by the right-hand member of the equation, in which the constants a, b, and c were determined by the different combinations of the four remaining facet numbers taken three at a time. The average values thus obtained were:

$$a = 1.363$$
$$b = 4.209$$
$$c = 0.276$$

By substituting these constants and the number of episomes, we may calculate the different facet numbers and extrapolate the facet numbers of hypothetical allelomorphs and their heterozygotes which have more than four episomes on the two protosomes of the female. The observed facet numbers and the calculated results are as follows:

Number of episomes in females	*Calculated facet number*	*Observed facet number*
0	373,250	740
1	335	325
2	48.2	49.0
3	28.3	28.1
4	24.4	24.5
5	23.4	——
6	23.2	——
7	23.1	——
8	23.1	——
∞	23.1	——

The calculated facet numbers fit the observed facet numbers very closely, except for full, the zero condition, which has already been pointed out as qualitatively different. This seems to substantiate the conclusion

reached earlier by the rates of mutation, namely, that the full gene consists of a protosome with no episomes.

The main point of this discussion of facet number is to bring out mathematically what seems evident from an inspection of the facet numbers, that is, that facet number in the bar series approaches a limit. This calculated limit is 23.1 facets. This formula has been applied to the corresponding facet numbers given by STURTEVANT (1925), also at 25°C. While his facet numbers run somewhat higher than those used here, the limit approached is almost identical, being 23.05 facets. Females homozygous for these hypothetical allelomorphs with three or more episomes on each protosome would not have a facet number appreciably different from homozygous ultrabar.

The genes of the infrabar series and facet number

The facet numbers given by STURTEVANT (1925) for the homozygotes and heterozygotes of the full, infrabar, and double-infrabar genes have been substituted in the same formula, and the constants calculated, as was done for the bar series. By using the four facet numbers determined by 1, 2, 3, and 4 infrabar episomes on the two protosomes of the female, and by extrapolating, it is evident that facet number in this series does not approach any positive number. The trend of the curve indicates that a female homozygous for an allelomorph with three infrabar episomes on each protosome would be almost, if not quite, without facets. If this were true, the threshold ratio of episomes to protosomes in this infrabar series would be 3:1. Assuming, as before, that flies with the same episome:protosome ratio have similar facet numbers, an allelomorph with six or more infrabar episomes would be completely dominant over full, and the flies would be without facets.

With all this discussion of hypothetical allelomorphs with more than two episomes, the reader may well wonder why they have not been found. STURTEVANT (1925), and the writer as well, have tried to find such allelomorphs without success. One may surmise either that the decrease in viability which accompanies decrease in facet number in these series may prevent the appearance of these more extreme forms, or else that the protosome at the bar locus is "saturated" by two episomes.

Other quantitative series with a completely dominant allelomorph

The bar series of allelomorphs, without having realized a completely dominant member, can be brought into harmony with other quantitative series, such as the albino allelomorphs in guinea pigs (WRIGHT 1925) or the white eye series in *Drosophila melanogaster* (MULLER 1920), each of which has a

completely dominant allelomorph. It is to be expected that in the bar series all allelomorphs with four or more episomes on each protosome would be *completely dominant* over the three known allelomorphs. The facet formula given above shows that there is a *threshold ratio of episomes to protosomes*, which is 2:1. Accordingly, there can be no change in facet number until this ratio goes below the threshold value. For example, mutation from a gene with four episomes to five episomes would go undetected either by a count of the facets of the homozygotes or of their heterozygotes with the known allelomorphs. On the other hand, two allelomorphs, one with three episomes, and another with four episomes, while indistinguishable in the homozygous condition, should be capable of separation by their heterozygotes with full because the episome:protosome ratio of the first is below the threshold of manifestation and the other is not.

The postulation of apparently identical end-members of a series, which differ only by their complete or incomplete dominance over the allelomorph at the other extreme of the series, has been realized in the vestigial series in *Drosophila melanogaster* reported by BRIDGES and MORGAN (1919). Here the four members allelomorphic to wild-type, named in the order of increasing wing reduction, are nick, antlered, strap, and vestigial. The nick homozygotes are indistinguishable from the wild-type homozygotes on the basis of somatic appearance; however, they can be separated by their dominance over vestigial, since the normal allelomorph is completely dominant while nick gives an intermediate compound with vestigial very much like the sex-linked character "notch."

The dominance of bar over infrabar

Using STURTEVANT'S (1925) facet counts of the different combinations of genes at the bar locus, WRIGHT (1929) pointed out ". . . that infrabar is virtually indistinguishable in its effects from bar in all combinations, as long as there is at least one true bar gene present, that is, is almost or quite completely recessive to bar, . . ." This dominance relation, as well as other evidence already brought out, shows that bar and infrabar are qualitatively different and throws some light in such other allelomorphic series as the eye and ocelli color series in the wasp, *Habrobracon juglandis* (A. R. WHITING 1926), in which there are three genes allelomorphic to the completely dominant wild-type, with its jet black eyes and ocelli. These mutant allelomorphs are: light ocelli, in which the eyes are black and the ocelli somewhat lighter; orange eyes and ocelli, which vary ". . . from very light cream to a deep red and from yellowish orange to pink"; and ivory eyes and ocelli, which are greenish white. These allelomorphs not only cannot be arranged

in any quantitative series but show further heterogeneity in that each is completely dominant over the ones following in the order named. This epistatic behavior is similar to that of bar and infrabar and makes it likely that these three mutations represent the loss (or, less probably, the gain) of three different kinds of episomes.

Other manifestations of mutation at the bar locus

As a test of the structure of the gene pictured in this paper, a study was made of other quantitative character changes accompanying mutation at the bar locus. On the basis of facet number, we arrange full, bar, and ultrabar eyes in a quantitative series in the order named. If other quantitative character changes caused by the same mutations gave reason for arranging these three in a different order, it would, obviously, be a serious criticism of the theory since it would indicate different structures for identical genes. An example of such other manifestations of these mutations is the increase in vigor accompanying a full mutation in a bar stock, where the full-eyed flies tend to crowd out the bar flies in a few generations. Likewise, bar mutants appearing in ultrabar stock crowd out the latter, which in turn are also crowded out if a full mutation occurs. Conversely, ultrabar flies never crowd out bar flies. This crowding out effect is obviously due to a variety of physiological differences affecting such things as number of eggs, length of immature period, and vigor. In a series of experiments designed to determine the quantitative relations of these other manifestations, flies of all possible combinations of the bar series were produced under uniform conditions at 25°C. The stocks used were forked ultrabar, forked bar, and forked full derived from the forked ultrabar by reverse mutation. The results are summarized in the following paragraphs.

While facet number may be regarded as a combined expression of the length and breadth of the eye, the width of the head gives an expression of the depth of the eye. The average widths of the heads of one hundred each of the six kinds of females and the three kinds of males are : Females—full homozygotes, 873 microns; full-bar heterozygotes, 760 microns; bar homozygotes, 681 microns; full-ultrabar heterozygotes, 658 microns; bar-ultrabar heterozygotes, 640 microns; ultrabar homozygotes, 637 microns. Males —full, 814 microns; bar, 636 microns; ultrabar, 601 microns.

It was noticed that ultrabar flies usually lack the median ocellus while the two lateral ocelli are unaffected. The median ocellus was measured by estimating the ratio of its diameter to the diameter of the lateral ocelli. These ratios were thrown into five classes ranging from that for equal-sized median ocelli down to zero, representing the absence of median ocelli. Flies

of the intermediate types occasionally had a double-appearing median ocellus, which recalls the double origin of this ocellus in development. The average of the two diameters of such ocelli were used. The average ratio of one hundred each of the six kinds of females and the three kinds of males are: Females—full homozygotes, 0.985; full-bar heterozygotes, 0.920; bar homozygotes, 0.623; full-ultrabar heterozygotes, 0.522; bar-ultrabar heterozygotes, 0.360; ultrabar homozygotes, 0.048. Males—full, 0.960; bar, 0.598; ultrabar, 0.042.

In connection with this work on the median ocellus it was noticed that the ultrabars often had 10 or more ocellar bristles while the fulls usually had 7 or 8. The average ocellar bristle numbers of one hundred each of the above-mentioned material are: Females—full homozygotes, 7.50; full-bar heterozygotes, 7.56; bar homozygotes, 8.13; full-ultrabar heterozygotes, 7.92; bar-ultrabar heterozygotes, 8.53; ultrabar homozygotes, 8.74. Males —full, 7.17; bar, 7.41; ultrabar, 9.11.

The percentage of sterile females also varies in the allelomorphs of the bar locus. Data on hand from large numbers of single pair matings and uniform culture methods indicate that about 10 percent of the full females, 30 percent of the bar females, and 90 percent of the ultrabar females are completely sterile.

It appeared in a number of experiments that the ultrabars were hatching somewhat later than other kinds. An experiment was carried out to find the differences in the time of emergence of the different combinations of full, bar, and ultrabar in females. An excellent control of this determination was obtained by mating each of the three kinds of heterozygotes to the three kinds of males. Each of these nine different matings gave two kinds of females and two kinds of males which developed in the same bottle from the same parents. The flies were cultured in 8-dram vials containing 9 cc of banana agar food at 25°C. Eight pairs of parents were used in each mating and transferred at three-hour intervals until they had laid eggs in a series of thirty bottles. The time of emergence was recorded at three-hour intervals throughout the hatching period. The average time of emergence of the full females was 218 hours after egg-laying. The average differences in hatching time as determined by this method, and their probable errors, are given in the following tabulation based on 2,013 females. Each number represents the mean difference in hours between the time of emergence of the female listed at the top of each column as compared with the one listed to the left when the two were produced in the same culture bottle and from the same parents at 25°C.

	full / bar	bar / bar	full / ultrabar	bar / ultrabar	ultrabar / ultrabar
full / full	+0.30±1.50		+4.08±1.04		
full / bar		−0.34±1.43	+2.02±1.39	+1.81±1.21	
bar / bar				+2.64±1.37	
full / ultrabar				+4.54±1.01	+10.10±2.57

These other somatic manifestations of mutation at the bar locus agree with facet number in that the values for bar are intermediate between those for full and ultrabar. The similarity of the full-ultrabar heterozygote to homozygous bar is consistent, and unless other experiments prove it untrustworthy, it provides a useful tool in working out the structure of the gene in other quantitative series of multiple allelomorphs. A comparison of the effects of these genes on these somatic characteristics shows nothing inconsistent with the view that they may accomplish their effects through a single intermediate agent, perhaps an enzyme. More critical evidence on this point should be furnished by a comparison of the effects of temperature on these different manifestations.

Dominance

At this point in the discussion it seems obvious that dominance, *per se*, is a property of the somatic effects of genes but not of their structure, since it not only varies from one manifestation to another but also depends upon the point of view with which we approach a single manifestation. Not only are the threshold ratios of episome number to protosome number different for these different manifestations, but some manifestations occur *above* certain episome:protosome ratios while others occur *below* certain other ratios. For example, variations in the median ocellus, which are parallel to variations in facet number, occur where the threshold of manifestation is below a 2:1 ratio; while variations in the number of ocellar bristles, which have an inverse relation to facet number, occur above a 0:1 ratio.

TWO DIFFERENT BAR DEFICIENCIES

A comparison of two cases of mutational phenomena involving a change at the bar locus substantiates the conclusion reached earlier in this paper

that the wild-type eye, as well as reverted bar, is represented by a gene at the bar locus. In the first of these cases (BRIDGES 1917) the bar locus of the deficient X-chromosome behaves like a full gene, indicating the presence of a protosome. In the other case (MORGAN 1927) the bar locus of the deficient X-chromosome does not behave like an allelomorph; that is, no protosome is present, and females are haploid for the bar locus. Tests showed that the latter case did not involve adjoining loci in the X-chromosome. From the evidence presented, no other interpretation of this case seems possible but that it was a "real" deficiency and that both the protosome and the bar episome were lost. In this case, the deficiency and a bar gene gave females with bar eyes; in BRIDGES' case, the deficiency and a bar gene gave females with eyes typical of full-by-bar heterozygotes.

The deficiency reported by BRIDGES (1917) occurred in a germ cell of a white bar male. It also produced changes at the forked and bar loci. In effect, it changed a bar gene to a full gene, and the normal gene for the forked locus to a forked gene. Forked mutations and similar allelomorphs of forked (MOHR 1922) have repeatedly arisen from the wild-type gene, with no reverse mutations reported. Differential rates of mutation in the two directions are interpreted, by methods discussed in connection with the bar series, to mean that the wild-type gene at the forked locus consists of a protosome with episomes attached in side-chain arrangement. Forked mutations and forked allelomorphs arise by the loss of all, or a part, of these side-chains. Applying this same interpretation to BRIDGES' forked-bar deficiency, we find that both the forked and bar loci lost episomes very much as if the genetic chromosome had been shaved of its side-chains for a short distance.

The same explanation may be used for other regional mutations such as the notch-8 deficiency described by MOHR (1923) which involved the loci for white, notch, and abnormal abdomen.

THE MUTABLE GENES OF *DROSOPHILA VIRILIS*

The migration of the episome on the chromosome

DEMEREC (1926, 1926a, 1927, 1928, 1929, 1929a) has found genes at three loci in the X-chromosome of *Drosophila virilis* which exhibit a higher rate of mutation than is known elsewhere in the animal kingdom. These genes arose by mutation from the wild-type, probably as single occurrences, and they frequently revert to the wild-type gene, depending on certain genetic factors in other chromosomes, crossing over, developmental stages, and age of the female. Under certain conditions 100 percent mutation is

realized. Each of these mutable genes has produced non-mutating genes which are indistinguishable in other respects from the parent genes. In all these mutable genes, there are differential rates of mutation in the two directions, with the high rate toward the wild-type indicating that the few original mutants resulted from additions of an episome and that their reversion represents its loss. Mutable reddish (body color) was the first to be found (DEMEREC 1928). In an experiment involving reddish, mutable miniature (wing character) was found (DEMEREC 1926a). The origin of mutable magenta (eye color) is not certain, as it may have gone unnoticed for several generations (DEMEREC 1927). These three mutable genes have so much in common in their mutational behavior that they may be regarded as resulting from the *same episome* acting at three loci on the X-chromosome.

The greater mutability of newly formed genes

From the evidence of chromosome maps, as well as the rates of mutation of genes under so-called natural conditions, it is evident that most genes are stable. Furthermore, the very numerous lethal mutations and the several cases of multiple allelomorphs wherein the greater rate of mutation is away from the wild-type indicate that most genes have side-chains of episomes. These old established genes seem to have achieved a degree of stability and a nicety of balance not found in genes formed by the recent additions of episomes. This is well borne out by the mutable genes of *Drosophila virilis* and by those at the bar locus in *D. melanogaster*, all of which represent additions to the germ-plasm.

The migration of the episome on the protosome

DEMEREC (1928) has supplied pertinent evidence on the process by which a rapidly mutating gene becomes stabilized. Reddish-alpha, immediately after its origin, was crossed repeatedly to yellow (a stable allelomorph) and gave a decreasing yield of reversions to wild-type. The reversion in the second generation was 12.4 percent; in the third generation, 1.6 percent; fourth, 0.6 percent; fifth, 0.32 percent; sixth, 0.043 percent; and in the seventh generation there were no reversions. On the basis of our side-chain explanation of gene mutation, such evidence is interpreted to mean that there are certain positions on a protosome where an episome will give a more stable gene than other positions. If it be supposed that the "equatorial" position is most unstable and that an episome varies somewhat in its position from one cell generation to another, then episomes at the "equator" would be eliminated most rapidly and those nearer the

"poles" of the protosome would be in a more "protected" position. When DEMEREC saw that the mutation rate was dropping rapidly, he attempted to bring back the original high rate by selecting for mutability. In this he was partly successful, being able to maintain an average level of reversion amounting to 2.9 percent. It is understandable that he should not attain the original rate of reversion (12.4 percent), if we suppose that the "pressure" of mutability soon reaches an equilibrium with the "pressure" of selection when such high actual rates of mutation are involved.

The explanation of decreasing mutation rate by migration of the episome toward one, or both, of the poles of the protosome is further clarified by DEMEREC'S (1928) data. He found that almost half of the reddish-alpha reversions are crossovers between yellow (stable allelomorph of reddish-alpha) and scute, a gene 0.6 unit away from the reddish locus. No reversions were crossovers between yellow and sepia, which is 2.2 units on the other side of reddish. The remainder of the reversions were about equally distributed between non-crossovers and crossovers in other parts of the X-chromosome. It seems clear that the episome migrated toward the scute pole of the protosome but not toward the sepia pole.

DISCUSSION

Attraction and repulsion phenomena

Since homologous chromosomes lie side by side during synapsis, each gene attracting its homologue in the other chromosome, and since most genes are identical with their homologues in paired chromosomes, we may conclude that like genes attract each other. In "quantitative" series of multiple allelomorphs which are interpreted to be due to varying numbers of one kind of episome, the mutation rates indicate that like episomes unite to form side-chains. In the composite bar-infrabar and infarbar-bar genes, the two different kinds of episomes seem to repel each other and they take up positions near the opposite poles of the protosome as shown in figure 2. The unusually high mutability of the reddish-alpha gene in heterozygotes and not in homozygotes also indicates attraction and repulsion.

The symmetry of genes

Since the data assembled here indicate that the gene is a three-dimensionally differentiated entity, there are certain corollaries which cannot be altogether neglected. For example, the ordinary assumption that genes "split" during cell division seems rather too naïve. General considerations of growth and symmetry phenomena among living things make it more rational to regard a gene, in its rôle of autocatalyst, as "regenerating" a

homologue by its side. In the same way this "regenerated" gene may also be expected to be a mirror image of its progenitor. A number of interesting analogies may be drawn between such "dextro" and "laevo" genes and optically active organic substances, but their genetic applications seem too problematical to discuss further.

WILLSTÄTTER'S structure of enzymes

I am indebted to Doctor A. H. HERSH for pointing out that the structure of the gene developed in this paper resembles in several points the structure of enzymes as given by WILLSTÄTTER (1926), who says: "The 'molecule' of an enzyme appears to consist of a colloidal carrier and a purely chemically active group. The specificity of the enzyme is vested wholly in this active group, which can apparently be transferred from one colloidal carrier to another. It has not yet been possible to separate the enzyme molecule proper from the colloidal carrier without loss of activity." Genes probably produce their effects through, and are the parent substances of, enzymes, so that it is fitting that they should be similar in structure.

Other structural explanations of gene mutation

When MAY (1917) reported the occurrence of reverse mutations of bar to full, he postulated the following explanation:

"If the normal wild fly carries a limiting factor with respect to the facet number then it is possible by partial non-disjunction for the factor to pass from one chromosome of a pair to the other, giving one chromosome without a limiting factor and the other with a double limiting factor. The bar-eyed race of Drosophila may be derived from such a chromosome with two limiting factors or factor groups, the mate of the chromosome having been lost in the maturation of the egg. If then in the bar-eyed race a second non-disjunction again separates the two factors the result should be one chromosome with triple factors and one heterozygous female. If the former passes into the egg it should give rise to a further reduction in the facet number, but it is possible that a fly with such a chromosome does not live. It is possible also that the male with 34 facets contained a chromosome with a triple reducing factor."

According to the mathematical relations among the members of the bar series developed in the present paper, ultrabar fulfills the above prediction, but the evidence from mutation rates and other data indicates that bar does not represent the doubling of an inhibiting factor already present in the full gene but rather the addition of a new factor (episome).

CORRENS (1919), in explaining variegation in certain plants, postulated

a structure in which he conceived of the gene as a large molecule with varying numbers of the same chemical radical attached, thus producing varying amounts of green and white in variegated foliage. The gene for intermediate variegation was held to have an intermediate number of radicals, to which new ones might be added or old ones sloughed off to produce plants with larger or smaller green or white areas. The addition of the greatest possible number of these chemical radicals or the loss of all of them, he inferred, would give a self-green plant or a self-white plant, one or both of which should be constant. As long as the number of radicals was intermediate, selection would be effective in either direction until one or the other extreme was reached.

The large and complicated series of multiple allelomorphs in the cob and pericarp colors of maize has been investigated by many workers. In a study of the mutational phenomena accompanying variegation, EYSTER (1924, 1925) has concluded that these genes consist of varying numbers of pigment-producing and non-pigment-producing *gene elements*. In an intermediate color, such as orange pericarp, he conceives that mitosis may divide the two elements of the gene unequally between the two daughter genes, so as to produce adjacent areas of lighter and darker color. The side-chain explanation can equally well account for the same facts by the gain or loss of episomes between the two daughter genes.

A rather superficial examination of the data and material suggests an explanation that is alternative to both CORRENS' and EYSTER's, namely, that there are varying numbers of one kind of episome determining the total amount of pigment present, and that another kind of episome causes the pigment present to be evenly distributed or, if it be lost, to be concentrated in certain areas. This latter phenomenon would not involve the unequal assortment of gene elements, but rather the critical concentrations of chromogenic substances unequally distributed during the ontogeny, very much as CONKLIN found among the organ-forming stuffs of Crepidula.

ACKNOWLEDGMENTS

This explanation of gene mutation was developed during 1923 and 1924 in the Zoological Laboratories of the UNIVERSITY OF ILLINOIS under the direction of Professor CHARLES ZELENY. Any contribution it may make to the literature of genetics is a reflection of the intimate association I have enjoyed with him during the past eleven years. I wish to thank him particularly for his stimulating criticism and for the repeated admonition that a hypothesis is valuable only in so far as it is capable of being tested.

Whatever clarity may attend the presentation of this paper is largely a result of the untiring efforts of my friend, Mr. H. CARL OESTERLING, editor for the ILLINOIS NATURAL HISTORY SURVEY.

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LITERATURE CITED

BRIDGES, CALVIN B., 1917 Deficiency. Genetics **2**: 445–465.

BRIDGES, C. B., and MORGAN, T. H., 1919 II. The second-chromosome group of mutant characters. Pub. Carnegie Instn. 278.

CORRENS, C., 1919 Vererbungsversuche mit buntblättrigen Sippen. I. *Capsella Bursa pastoris* albovariabilis und chlorina. Sitzungsberichte der Akademie der Wissenschaften zu Berlin. **33**: 585–610.

DEMEREC, M., 1926a Reddish—A frequently "mutating" character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **12**: 11–16.

1926b Minature—A second frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **12**: 687–690.

1927 Magenta-alpha—A third frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **13**: 249–253

1928 Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. Genetics **13**: 359–388.

1929a Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **15**: 834–839.

1929b Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **15**: 870–876.

DRIVER, ERNEST C., 1926 The temperature-effective period—the key to facet number in Drosophila. J. Exp. Zool. **46**: 317–332.

EYSTER, WILLIAM H., 1924 A genetic analysis of variegation. Genetics **9**: 372–404.

1925 Mosaic pericarp in maize. Genetics **10**: 179–196.

HANSON, FRANK BLAIR, 1928a The effect of X-rays in producing return gene mutations. Science **67**: 562–563.

1928b The effects of X-rays on productivity and the sex ratio in *Drosopila melanogaster*. Amer. Nat. **62**: 352–361.

1929 Duration of effects of X-rays on male germ cells in *Drosophila melanogaster*. Amer. Nat. **63**: 511–516.

HERSH, A. H., 1922 The effect of temperature upon the heterozygotes in the bar series of Drosophila. Thesis, Univ. Illinois Grad. School (not published).

1924 The effect of temperature upon the heterozygotes in the bar series of Drosophila. J. Exp. Zool. **39**: 55–71.

1929 The effect of different sections of the X-chromosome upon bar eye in *Drosophila melanogaster*. Amer. Nat. **63**: 378–382.

HERSH, ROSELLE KARRER, 1924, The effect of temperature upon the full-eyed race of Drosophila. J. Exp. Zool. **39**: 43–53.

KRAFKA, JOSEPH, JR., 1920 The effect of temperature upon facet number in the bar-eyed mutant of Drosophila. J. Gen. Physiol. **2**: 445–464.

LUCE, WILBUR M., 1926 The effect of temperature upon infrabar, an allelomorph of bar eye in Drosophila. J. Exp. Zool. **46**: 301–316.

MAY, H. G., 1917 Selection for higher and lower facet numbers in the bar-eyed race of Drosophila and the appearance of reverse mutations. Biol. Bull. **33**: 361–395.

MOHR, O. L., 1922 Cases of mimic mutations and secondary mutations in the X-chromosome of *Drosophila melanogaster*. Z. indukt. Abstamm.- u. VererbLehre., **28**: 1–22.

1923 A genetic and cytological analysis of a section deficiency involving four units of the X-chromosome in *Drosophila melanogaster*. Z. indukt. Abstamm.- u. VererbLehre. **32**: 108–233.

MORGAN, T. H., 1927 Exceptional classes of individuals in an experiment involving the bar locus of Drosophila. Hereditas **9**: (Festskrift för W. Johannsen) 1–9.

MULLER, H. J., 1920 Further changes in the white-eye series of Drosophila and their bearing on the manner of occurrence of mutation. J. Exp. Zool. **31**: 443–473.

STURTEVANT, A. H., 1925 The effects of unequal crossing over at the bar locus in Drosophila. Genetics **10**: 118–147.

1928 A further study of the so-called mutation at the bar locus of Drosophila. Genetics **13**: 401–409.

STURTEVANT, A. H., and MORGAN, T. H., 1923 Reverse mutation of the bar gene correlated with crossing over. Science **57**: 746–747.

THOMPSON, DAVID H., 1921 A new type of sex-linked lethal in Drosophila. Anat. Rec. **20**: 215.

1925 Evidence of structure in the gene. Amer. Nat. **59**: 91–94.

TICE, SABRA COLBY, 1914 A new sex-linked character in Drosophila. Biol. Bull. **26**: 221–230.

WHITING, ANNA R., and BURTON, RAYMOND H., 1926 Quadruple allelomorphs affecting eye-color in Habrobracon. Amer. Nat. **60**: 285–290

WILLSTÄTTER, R., 1926. Chemical progress. Chem. Soc. of London, Rep. Prog. Chem. 232–234.

WRIGHT, SEWALL, 1925 The factors of the albino series of guinea pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

1929 The dominance of bar over infra-bar in Drosophila. Amer. Nat. **63**: 479–480.

ZELENY, CHARLES, 1920a A change in the bar gene of *Drosophila melanogaster* involving further decrease in facet number and increase in dominance. J. Exp. Zool. **30**: 293–324.

1920b The tabulation of factorial values. Amer. Nat. **54**: 358–362.

1921 The direction and frequency of mutation in the bar-eye series of multiple allelomorphs of Drosophila. J. Exp. Zool. **34**: 203–233.

1922 The effect of selection for eye facet number in the white bar-eye race of *Drosophila melanogaster*. Genetics **7**: 1–115.

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INTERNATIONAL CONGRESS FOR STUDIES REGARDING POPULATION

Organized by the Italian Committee for the Study of Population Problems, an International Congress for Studies Regarding Population, will be held in Rome from 7th to 10th September next, under the Honorary Chairmanship of H. E. BENITO MUSSOLINI and the effective Chairmanship of Professor CORRADO GINI, President of the aforesaid Italian Committee. The Congress will include: (a) An Honorary Committee. (b) An International Committee of Patronage, composed of presidents and representatives of national scientific organizations for the study of population problems, and other persons. (c) An Organizing Committee, composed of Italian members will be entrusted with the organization of the Congress and will be responsible for the acceptance of communications.

The program of the Congress is scientific, so that political, moral, or religious propaganda is forbidden, even if questions concerning the problems of population are involved.

The Congress will consist of the following Sections: Biology and Eugenics, Anthropology and Geography, Hygiene and Medicine, Demography, Economy, Sociology, History, Methodology. English, French, German, Italian and Spanish, will be the official languages of the Congress.

All taking part in the Congress will be entitled to send communications of which the final text must reach the Organizing Committee before July 1st. It is advisable to send as soon as possible the subjects of the communications with a short statement of their contents to the Organizing Committee.

Some subjects of special importance, chosen by the Organizing Committee, will be the object of reports by persons previously nominated.

The fee entitling members to take part in the Congress and to receive a copy of the Proceedings is: 50 lire for those invited and 100 Lire for all others.

A more detailed program will be distributed, containing the titles of the reports together with the names of the reporters and of the members of the Honorary Committee, the International Committee of Patronage and Organizing Committee.

All those who are interested in the Congress and would like to receive the detailed program are invited to apply to the "Comitato Italiano per lo Studio dei Problemi della Popolazione"—10 Vie delle Terme di Diocleziano, Roma (Italy).

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